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ENZYMATIC ASPECTS OF HOST-PARASITE INTERACTIONS

I. An Endo-Polygalacturonate *Trans*-eliminase Produced by *Phytophthora capsici* and its Effects on Plant Tissues[1,2]

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Abstract

Lesion extracts of *Phytophthora capsici*-infected egg plant fruits contained a heat stable endo-polygalacturonate *trans*-eliminase. This enzyme has a pH optium at 8.5, Ca^{2+} requirement and substrate preference for polygalacturonic acid. It was purified to 600-fold from crude lesion extract by the system of ammonium sulfate fractionation, DEAE cellulose column chromatography, and isoelectric focusing. The molecular weight of the enzyme was estimated to be 96,000 and it has an isoelectric point of pH 8.9. The purified endo-polygalacturonate *trans*-eliminase, free from proteinase, phosphatidase, polygalacturonase, pectin methyl *trans*-eliminase as well as hemicellulase, was used for study on its effects on plant tissues. Results indicated that no detectable maceration and cellular death of egg plant discs treated with the purified enzyme was found after 30 min treatment, while drastically changed in both processes occurred after 60 min, and it was completed at 120 min. Similar results were obtained when potato discs were treated with the same enzyme. Surprisingly, both potato and egg plant discs treated with the purified enzyme failed to induce electrolyte leakage, and there were no significant differences in conductance as compared with buffer and autoclaved enzyme controls. The fact that *P. capsici* endo-polygalacturonate *trans*-eliminase could not induce permeability change in the plant tissues was not elucidated.

Introduction

Studies on the role of pectic enzymes in plant diseases have been mainly concerned with soft rot and wilt diseases. Several lines of evidence have demonstrated that pectic enzymes which obtained from diseased tissues and cultural filtrates are responsible for tissue degradation. The most convincing

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instances are the purified enzymes such as endo-polygalacturonate *trans*-eliminase (Mount *et al.*, 1970; Garibaldi and Bateman, 1971; Tseng and Mount, 1974; Basham and Bateman, 1975; Gardner and Kado, 1976), endo-polygalacturonase (Ayers *et al.*, 1969; Kaji and Okada, 1969; Wang and Keen, 1970) and endo-pectin methyl *trans*-eliminase (Byrde and Fielding, 1968; Ohuchi and Tominaga, 1974; Plumbley and Pitt, 1979) which can cause tissue maceration in various plants.

Phytophthora capsici Leonian is an aggressive pathogen of many crops, particularly attacking a wide variety of plants such as Solanaceae and Cucurbitaceae in tropics and subtropics. The disease is characterized by browning and rotting of fruits, stems as well as roots. The rapidity which this pathogen can destroy its susceptible host tissues in indicated of its ability to produce large quantities of a number of enzymes during pathogenesis. Among them pectic enzymes have been considered to play an important role in disintergration of host tissues, however, little information is available on the research of pectic enzymes which induced by the pathogen during disease development.

This report deals with the purification of an endo-polygalacturonate *trans*-eliminase produced by *P. capsici* and the characteristics of this enzymes in terms of its ability for inducing tissue maceration, electrolyte loss and killing of plant cells.

Materials and Methods

The isolate of *Phytophthora capsici* Leonian used for this study was kindly obtained from my colleague Dr. H. S. Chang. Stock cultures were maintained on potato dextrose agar at 26°C, and transfers were made at 1-month intervals.

Preparation of endo-polygalacturonate trans-eliminase

Market available egg plant fruits were surface disinfected by 70% alcohol for 5 min and inoculated with mycelial mass of *P. capsici*, and then transfered at 28°C for a week. After incubation, the lesion areas of rotted fruits were removed and extracted with one volume of distilled water by grinding for 1 min in a Virtis '45' homogenizer at high speed. The extracts were filtered through four layers of cheesecloth to remove debris and then centrifuged at 20,000 g for 20 min at 4°C. The supernatants were immediately served as crude enzyme sources.

Enzyme assay

Endo-polygalacturonate *trans*-eliminase (endo-PGTE) activity was measured by the periodate-thiobarbituric acid (TBA) method (Preiss and Ashwell, 1963),

by the increase of reducing group of reaction mixtures (Nelson, 1944), or by viscosity loss of sodium polypectate (Garibaldi and Bateman, 1971). Reaction mixtures assayed by the thiobarbituric acid and reducing group procedures contained 0.1 ml of 0.6% sodium polypectate in 0.05 M Tris-HCl buffer (pH 8.0) plus 10^{-4} M $CaCl_2$ and 0.1 ml enzyme at 30°C for 1 hr. Viscosity loss was estimated with size 300 Fenske-Ostwald viscometers containing 4.0 ml of 0.6 ml sodium polypectate buffered with 0.05 M Tris-HCl at pH 8.0, 1.0 ml of enzyme, and 2.0 ml of water. Measurement were made at 30°C (Bateman, 1966). A unit of endo-polygalacturonate *trans*-eliminase activity was defined as that amount of enzyme giving an increase in absorbance of 0.1 in 60 min at 548 nm in the thiobarbituric acid assay.

All of the controls contained autoclaved enzymes instead of active enzymes. Protein concentration was estimated by the method of Lowry *et al.* (1951).

Enzyme Purification

Crude enzyme extract (250 ml) was brought to 20% saturation with powdered ammonium sulfate, allowed to stand for 1 hr at 4°C, and centrifuged for 20 min at 20,000 g. The precipitated was collected and dissolved in 10 ml of distilled water. This procedure was repeated for ammonium sulfate saturation to 40, 60, 80 and 95%. The precipitate of each fraction was immediately assayed for protein content and endo-polygalacturonate *trans*-eliminase activity.

The 40–60% ammonium sulfate fraction which contained a bulk of endo-polygalacturonate *trans*-eliminase was applied to a 1.6×56 cm column of diethylaminoethyl cellulose (DEAE cellulose, Cl^- form) buffered with 0.05 M Tris-HCl buffer at pH 8.0. The column was eluated with 30 ml of the Tris-HCl buffer followed by a NaCl gradient in the buffer until 0.4 M NaCl was reached. Five-ml fractions were collected and assayed for the enzyme activity.

In the current study, although two endo-polygalacturonate *trans*-eliminase peaks were obtained by fractionation on DEAE cellulose column (Fig. 3), only the major peak fractions (No. 22–25) were combined and dialyzed for 24 hr against several liters of distilled water. This enzyme fraction was then subjected to isoelectric focusing in a LKB 8101 Ampholine electrofocusing apparatus equiped with a 110-ml column (LKB Produkter AB, Bromma, Sweden) containing a pH 7-10 Ampholine carriers. Electrofocusing was carried out at 4°C for 48 hr, at that time the pH gradient had formed and the current had stabilized (500 v, 0.9 ma). Five-ml fractions were collected at a flow rate of 2 ml/min, and the pH of each fraction was measured. Each fraction was dialyzed against distilled water at 4°C for 24 hr and then assayed for endo-polygalacturonate *trans*-eliminase activity as well as protein content. This

procedure was served to determine the isoelectric point of the enzyme and as the final step of its purification.

Estimation of the molecular weight of endo-polygalacturonate trans-eliminase

The molecular weight of endo-polygalacturonate *trans*-eliminase was estimated by gel filtration using Sephadex G-75 in the manner described by Andrews (1964) and by SDS-acrylamide gel eletrophoresis (Weber and Osborn, 1969). Blue dextran (mol. wt. 2,000,000), crystalline bovine serum albumin (mol. wt. 67,000), dimer cytochrome C (mol. wt. 26,000) and cytochrome C (mol. wt. 13,000) were used as reference standards in the gel filtration experiments.

Measurements of permeability change, tissue maceration and cellular death

The permeability change of plant tissue treated with purified endopolygalacturonate *trans*-eliminase was measured by determining the increase in conductivity of the bath solution. Reaction mixtures which contained 15 discs of potato (10 mm diam × 1 mm thick) or egg plant (9 mm diam × 2 mm thick) in 2.0 ml of the purified enzyme plus 8.0 ml of 0.01 M Tris-HCl buffer pH 8.0 in a 15 ml test tube at 30°C. The conductivity of the solution was measured at zero time and at 30-minute intervals with a conductivity bridge (YSI model 31 conductivity Bridge, Yellow Springes Instrument Co.)

Tissue maceration was evaluated and rated as described by Bateman (1963) and Mount *et al.* (1970). Discs were placed in enzyme preparations as described above, buffered at pH 8.0 with 0.05 M Tris-HCl buffer. Measurements of tissue maceration were made over 3-hr incubation period with endo-polygalacturonate *trans*-eliminase. Discs which did not macerate received a rating of 0, while those that were completely macerated received a score of 5. Cellular death was determined by using the method of Tribe (1955), in which the degree of retention of neutral red within the discs (cells or protoplasts) was a measurement of cellular death. Reaction mixtures were the same as those described for tissue maceration. Cells (protoplasts) which retained the dye were considered alive and were scored O. Cellular death was indicated by the absence of stained protoplasts and these discs received a rating of 5. Intermediate values were assigned to discs which exhibited various degrees of maceration and neutral red retention.

Results

Crude enzyme preparations from *P. capsici*-infected egg plant fruits contained active endo-polygalacturonate *trans*-eliminase as determined by TBA assay (Preiss and Ashwell, 1963), Nelson's test (Nelson, 1944) and Viscometric method (Garibaldi and Bateman, 1971). Assays for healthy egg plant fruit

tissues and potato tubers did not contain detectable endo-polygalacturonate *trans*-eliminase. The optimum pH of the enzyme was at pH 8.5, and no activity was detected in either enzyme source at pH values lower than pH 7.0 (Fig. 1).

The thermostability of the crude endo-polygalacturonate *trans*-eliminase from lesion extracts was examined by incubating 10-ml samples that had been dialyzed overnight against several hundred volumes of distilled water in a water both at 10-80°C at 10°C intervals for 30 min. Enzyme incubated at 0°C served as the control. After the temperature treatments, assays were made by TBA method. There was no significant loss in endo-polygalacturonate *trans*-eliminase activity in the 10, 20, 30, and 40°C treatments as compared to the control. However, the enzyme activity was declined at 50°C and was completely inactivated at 60°C (Fig. 2).

Purification of P. capsici endo-polygalacturonate trans-eliminase

Crude lesion extracts of *P. capsici*-infected egg plant fruits were used for this purpose. Two hundred fifty milliters of crude enzyme was dialyzed overnight against distilled water at 4°C. This enzyme preparation contained 0.165 mg protein/ml and a specific activity of 0.48 (Table 1).

The dialyzed enzyme was then subjected to $(NH_4)_2SO_4$ fractionaton. Maximum endo-polygalacturonate *trans*-eliminase activity was found in the precipiate obtained between 40 and 60% saturation and had a specific activity

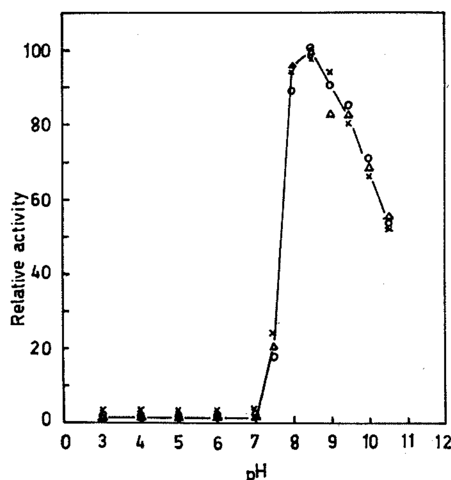

Fig. 1. The effect of pH on endo-polygalacturonate *trans*-eliminase activity in crude lesion extracts of ***Phytophthora capsici***. Endo-PGTE activity was determined by the reducing group (0), thiobarbituric acid (△) and viscosity loss (×) method.

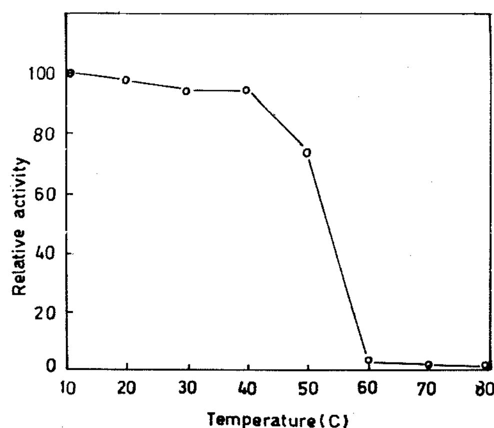

Fig. 2. Thermal stability of endo-polygalacturonate *trans*-eliminase upon exposure to the indicated temperatures for 30 min. Crude lesion extracts of ***Phytophthora capsici*** was used. Endo-PGTE activity was assyed by TBA method.

Table 1. *Purification of* ***Phytophthora capsici*** *endo-polygalacturonate* ***trans****-eliminase*

Step	Volume (ml)	Total protein (mg)	No. of units of activity[b]	Specific activity (units/mg)	Yield (%)	Purification (fold)
Crude enzyme[a]	250	41.25	20	0.48	100	—
40-60% $(NH_4)_2SO_4$	10	0.73	8.87	12.15	443	25.3
DEAE cellulose Chromatography	15	0.047	4.66	99.15	23.3	206.5
Isoelectric focuing	15	0.025	7.2	288	36.0	600

[a] Crude enzyme was obtained from lesion extracts of ***P. capsici***-infected egg plant fruits at 28°C for a week.

[b] A unit of the enzyme activity was defined as that amount of enzyme giving an increase in absorbance of 0.1 in 60 min at 548 nm in TBA assay.

of 12.15. Thus, a 25.3-fold purification was achieved by this two-step procedure.

For further purification of the endo-polygalacturonate *trans*-eliminase, the $(NH_4)_2SO_4$ fraction (40–60%) which contained a bulk of the enzyme was subjected to a DEAE cellulose column. Results (Fig. 3) indicated that two endo-polygalacturonate *trans*-eliminase peaks were obtained in the column. However, in this study, only the major peak fractions (No. 22–25) were pooled and assayed for the enzyme activity. The specific activity of this partly purified enzyme was 99.15 and it had been purified to 206.5-fold. This partly purified enzyme was dialyzed and subjected to isoelectric focusing by using a Ampholine carries with a pH range of 7–10. The enzyme was finally purified to 600-fold and the isoelectric point (pI) of the enzyme was estimated to be

pH 8.9 by this procedure. A summary of the purification of *P. capsici* endo-polygalacturonate *trans*-eliminase was illustrated in Table 1.

The molecular weight of this purified endo-polygalacturonate *trans*-eliminase was estimated to be 96,000 by gel filtration on Sephadex G-75 column (Fig. 5) and also further confirmed by SDS-acrylamide gel electrophoresis.

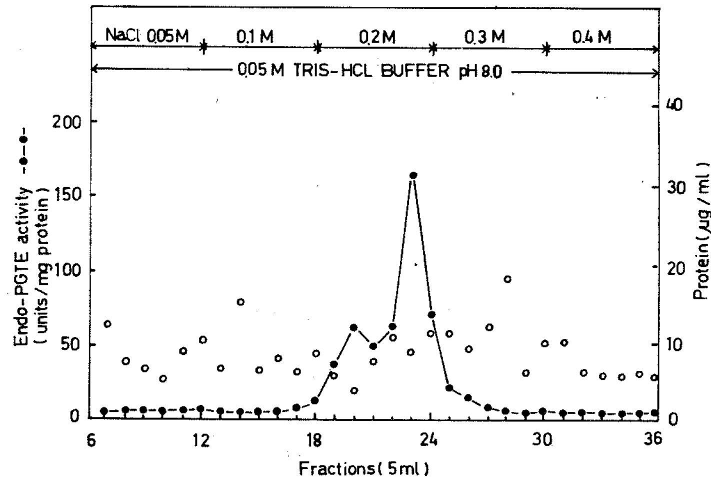

Fig. 3. Elution profile of endo-polygalacturonate *trans*-eliminase in an ammonium sulfate (40-60%) fraction of lesion extracts of ***Phytophthora capsici*** by DEAE cellulose column chromatography. Ten-ml of the enzyme was applied to a 1.6×56.0 cm column. Five-ml fractions were collected and endo-PGTE activity was determined by TBA method.

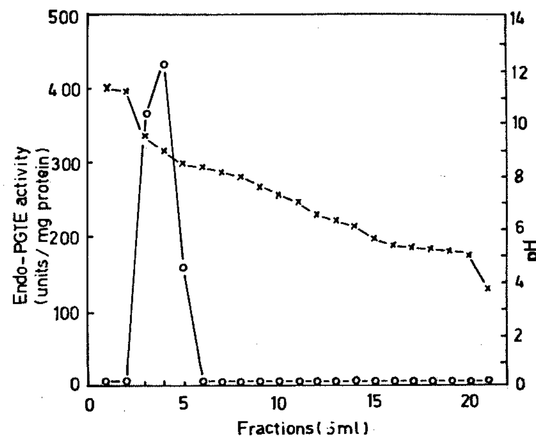

Fig. 4. Isoelectric focusing of 15 ml of dialyzed endo-polygalacturonate *trans*-eliminase from DEAE cellulose column (Fig. 3, fraction No. 22-25). A pH range of 7-10 Ampholine carriers was used. Five-ml fractions were collected and the enzyme was assayed by TBA method.

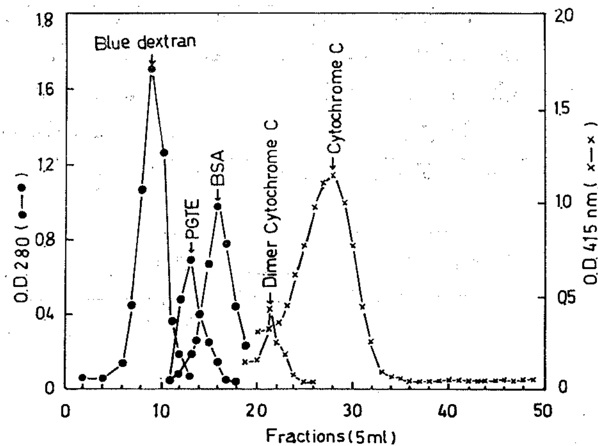

Fig. 5. Molecular weight of endo-polygalacturonate *trans*-eliminase estimated by Sephadex G-75 column. Maker proteins: bovine serum albumin (BSA, mol. wt. 67,000), dimer cytochrome C (mol. wt. 26,000) and cytochrome C (mol. wt. 13,000). The void volume of this column was determined by using blue dextran. Endo-PGTE activity was assayed by TBA method.

Tissue maceration, cellular death and electrolyte leakage by purified endo-polygalacturonate ***trans*-*eliminase produced by*** ***P. capsici***

Experiments were made with the combined fractions (No. 3–5) from an electrofocusing column (Fig. 4) which contained purified endo-polygalacturonate *trans*-eliminase but free from proteinase, phosphatidase, peroxidase, and enzymes which attack hemicellulose at pH 8.0 to determine the relationship between electrolyte leakage, maceration, and cellular death with plant tissues. Discs of egg plant fruits were treated with the purified enzyme, autoclaved enzyme, and buffer alone. Result (Fig. 6B) indicated that endo-polygalacturonate *trans*-eliminase failed to induce electrolyte leakage of egg plant tissue within a 210-min periods. Maceration and cellular death of egg plant discs were no detectable after 30 min treatment, however, drastically changed in both processes were found after 60 min, and it was completed after 120 min. There was no indication of either maceration or cellular death in autoclaved enzyme control (Fig. 6A).

When potato discs were used for the similar experiments, again, the purified endo-polygalacturonate *trans*-eliminase failed to induce electrolyte loss in potato tissues, and there were no significant differences in conductance as compared with buffer and autoclaved enzyme treatments (Fig. 7B). Tissue maceration and cellular death paralleled each other when potato discs were treated with the purified endo-polygalacturonate *trans*-eliminase after 90 min, however, no detectable tissue maceration and cellular death of potato discs

which treated with autoclaved enzyme has been found even after 180 min incubation (Fig. 7A).

Discussion

An endo-polygalacturonate *trans*-eliminase has been isolated from *P. capsici* with egg plant tissues, and it was purified to 600-fold. This enzyme has a pH optimum at 8.5, Ca^{+2} requirement, and substrate preference for polygalacturonic acid. It is similar to those partial purified *trans*-eliminase of certain

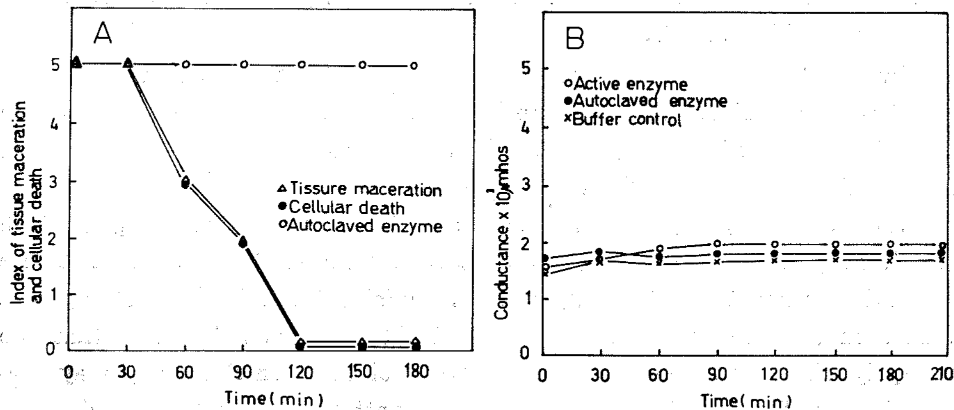

Fig. 6. A) Maceration and cellular death of discs of egg plant fruits treated with purified endo-polygalaturonate *trans*-eliminase. Reaction mixtures contained 2ml of enzyme, 8 ml of 0.05 M Tris-HCl buffer (pH 8.0), and 15 egg plant discs (9 mm dia × 2 mm thick) in 15 ml test tube at 30°C. Index of tissue maceration and cellular death (0→no maceration or cellular death; 5→completed maceration or cellular death). B) Electrolyte leakage from egg plant discs after treatment with purified endo-PGTE, reaction mixture same as (A).

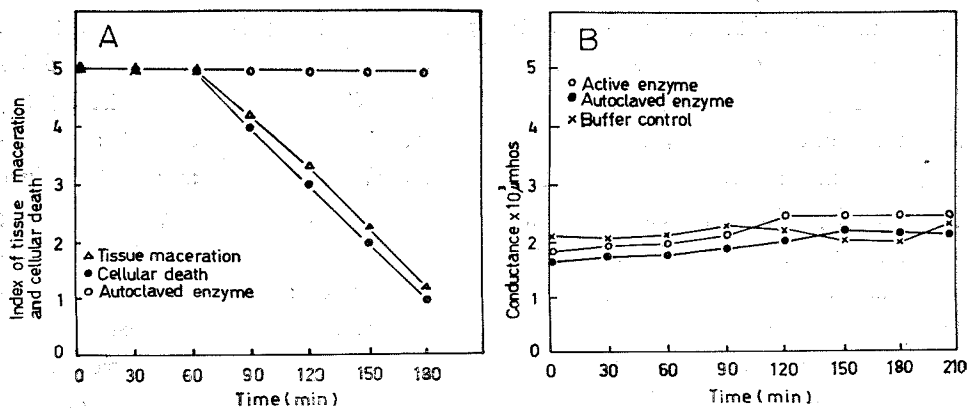

Fig. 7. A) Maceration and cellular death of potato discs (10mm dia × 1mm thick) treated with purified endo-polygalaturonate *trans*-eliminase, reaction mixture same as Fig. 6, except potato discs were used. B) Electroyte leakage from potato discs after treatment with the purified endo-PGTE, reaction mixture same as described above.

bacteria (MacMillan and Vaughn, 1964; Garibaldi and Bateman, 1970; Ohuchi and Tominaga, 1974; Tseng and Mount, 1974; Basham and Bateman, 1975) and differs from those of oligogalacturonate *trans*-eliminase, which have a neutral pH optimum (Moran *et al*.. 1968; Hatanaka and Ozawa, 1970). The isoelectric point (pI=8.9) is lower than that of endo-polygalacturonate *trans*-eliminase of *Erwinia carotovora* (Tseng and Mount, 1974). The molecular weight of this enzyme was estimated to be 96,000. This is rather high as compared with polygalacturonic acid *trans*-eliminases (mol. wt. 30,000 to 36,000) of *E. Chrysanthemi* (Garibaldi and Bateman, 1971) and *E. rubrifaciens* (mol. wr. 41,000) (Garnder and Kado, 1976).

Two polygalacturonate *trans*-eliminase fractions were eluted from the DEAE cellulose column (Fig. 3). Both polygalacturonate *trans*-eliminases were determined to be of endo-type by using viscometric assay and TBA method. The reaction products in the viscosity assay absorbed a maximal light at 230 nm when sodium polypectate was used as substrate; a red chromogen which was formed with TBA and products gave a wavelength at 548 nm. These results illustrated the pectic enzyme of *P. capsici* in the lesion extracts appears to be a endo *trans*-eliminative cleavage of the substrate. The fact that two endo-polygalacturonate *trans*-eliminases were obtained in this study may suggest that two "isozyme" were induced by the pathogen during disease development. Garibaldi and Bateman (1971) has demonstrated the presence of multiple polygalacturonic acid *trans*-eliminases in cultural filtrate of *E. Chrysanthemi*, and recently, Cervone *et al*. (1978) reported two polygalacturonase isozymes from *Rhizoctonia fragariae* showed similar amino acid compositions.

Increased loss of electrolytes by tissue invaded by plant pathogens has been observed by a number of investigators for a diverse group of diseases (Williams *et al*., 1967; Lai *et al*., 1968; Hancock, 1968; Mount *et al*., 1970). This is evidence that membrane permeability changes can be induced by toxins of pathogen origin as well as enzymes (Samadder and scheffer, 1968; Bashman and Bateman, 1975). Mount *et al*. (1970) indicated that an endo-polygalacturonate *trans*-eliminase produced by *E. carotovora* was able to induce permeability change, tissue maceration and cellular death of potato tissue; furthermore, Tseng and Mount (1974) studied on the toxicity of endo-polygalacturonate *trans*-eliminase, phosphatidase and protease to potato and cucumber tissues revealed that only endo-polygalacturonate *trans*-eliminase induced leakage of ^{86}Rb from potato discs, but no leakage of ^{86}Rb occurred when cucumber tissues treated with any of the purified enzymes. In the current study, similar results were found when egg plant and potato discs treated with purified *P. capsici* endo-polygalacturonate *trans*-eliminase, no appreciable electrolyte leakage in both tissues, however, tissue maceration and cellular

death can be caused by the same enzyme. The fact that the purified *P. capsici* endo-polygalacturonate *trans*-eliminase failed to induce permeability in its host tissues are still unknown. It would be interesting to find out what is the mechanism(s) behind it, and this needs for further investigation.

Recently, a non-pectolytic protein from *P. capsici* that macerates cucumber tissue has been isolated by Yashikawa *et al.* (1977). The maceration factor has been determined as a heat stable protein, possible an enzyme. Whether it will cause permeability and cellular death in its host tissues has not been studied. In our lesion extracts of *P. capsici*, however, consisted of a consider amount of macerating factor (endo-polygalacturonate *trans*-eliminase) which mainly caused tissue maceration of either potato or egg plant. Whether in our crude lesion extracts which also exist non-pectolytic protein (a macerating factor) is of interest to elucidate.

Literature cited

Andrews, P. 1964. Estimiation of the molecular weights of proteins by Sephadex gel-filtration. Biochem. J. **91**: 222–233.

Ayers, W.A., G.C. Papavizas and R.D. Lumsden. 1969. Purification and properties of the endopolygalacturonase of ***Aphanomyces euteiches.*** Phytopathology **59**: 929–930.

Basham, H.C. and D.F. Bateman. 1975. Killing of plant cells by pectic enzymes: the lack of direct injurious interaction between pectic enzymes or their soluble reaction products and plant cells. Phytopathology **65**: 141–153.

Bateman, D.F. 1963. The macerating enzyme of ***Rhizoctonia solani.*** Phytopathology **53**: 1178–1186.

Bateman, D.F. 1966. Hydrolytic and trans-eliminative degradatiom of pectic substances by extracellular enzymes of ***Fusarium solani*** f. ***phaseoli.*** Phytopathology **56**: 238–244.

Byrde, R.J.W. and A.H. Fielding. 1968. Pectin methyl-***trans***-eliminase as the maceration factor of ***Scleratinia fructigena*** and its significance in brown rot of apple. J. of General Microbiology **52**: 287–297.

Cervone, F., A. Scala and F. Scala. 1978. Polygalacturonase from ***Rhizoctonia fragariae:*** further characterization of two isoenzymes and their action towards strawberry tissue. Physiological plant Pathology **12**: 19–26.

Gardner, J.M. and C.I. Kado. 1976. Polygalacturonic acid ***trans***-elimenase in the osmotic shock fluid of ***Erwinia rubrifaciens:*** Characterizaton of the purified enzyme and its effect on plant cells. J. of Bacteriology **127**: 451–460.

Garibaldi. A. and D.F. Bateman. 1970. Association of pectolytic and cellulolytic enzymes with bacterial slow wilt of carnation caused by ***Erwinia chrysanthemi*** Burkh., McFad. et Dim. Phytopathology Mediters. **9**: 136–144.

Garibaldi, A. and D.F. Bateman. 1971. Pectic enzymes produced by ***Erwinia chrysanthemi*** and their effects on plant tissue. Physiological Plant Pathology **1**: 25–40.

Hancock, J.G. 1968. Effect of infection by ***Hypomyces solani f.*** sp. ***cucurbitae*** on apparent free space, cell membrane permeability, and respiration of squash hypocotyls. Plant physiol **43**: 1666–1672.

Hatanaka, C. and J. Ozawa. 1970. An oligogalacturonate transeliminase from ***Erwinia aroideae***. Agr. Biol. Chem. **34**: 1618–1624.

Kaji, A. and T. Okada. 1969. Purification and properties of an unusually acid-stable endopolygalacturonase produced by ***Corticium rolfsii.*** Archives of Biochemistry and Biophysics **131**: 203–209.

Lai, M.T., A.R. Weinhold and J.G. Hancock. 1968. Permeability changer in ***Phaseolus aureus*** associated with infection by ***Rhizoctonia solani***. Phytopathology **58**: 240-245.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**: 265-275.

MacMillan, J.D. and R.H. Vaughn. 1964. Punification and properties of a polygalacturanic acid-***trans***-eliminase produced by ***Clostridium multifermentans***. Biochemistry **3**: 564-572.

Moran, F., S. Nasuno and M.P. Starr. 1968. Olihogalacturonide ***trans***-eliminase from ***Erwinia carotovora***. Arch. Biochem. Biophys. 125- **734**: 741.

Mount, M.S., D.F. Bateman and H.G. Basham. 1970. Induction of electrolyte loss, tissue maceration, and cellular death of potato tissue by an endopolygalacturonate ***trans***-eliminase. Phytopathology **60**: 925-931.

Nelson, N. 1944. A photometric adaptation of the Somogui method for the determination of glucose. J. Biol. Chem. **153**: 375-380.

Ohuchi, A. and T. Tominaga. 1974. Pectin ***trans***-eliminase as a macerating factor of soft rot pseudomonas. Ann. Pytopathol. Soc. Japan **40**: 22-29.

Plumbley, R.A. and D. Pitt. 1979. Purification and Properties of a pectin methyl trans-eliminase, a phospholipase and two aminopeptidases produced in culture by ***Phoma medicaginis*** var. ***pinodella***. Physiological plant pathology **14**: 313:328.

Preiss, J. and Ashwell. 1963. Polygalacturonic acid metabolism in bacteria I. Enzymatic formation of 4-deoxy-L-threo-5 hexoseulose Uronic acid. J. Biol. Chem. **238**: 1571-1576.

Samaddar, K.R. and R.P. Scheffer. 1968. Effect of the specific toxin in ***Helminthosporium victoriae*** on hort cell membranes. Plant Physiol. **43**: 21-28.

Tseng, T.C. and M.S. Mount. 1974. Toxicity of endopolygalacturonate ***trans***-eliminase, phosphatidase and protease to patato and cucumber tissue. Phytopathology **64**: 229-236.

Tribe, H.T. 1955. Studies in the physiology of parasitism. XIX. On the killing of plant cells by enzymes from ***Botrytis cinerea*** and ***Bacterium aroideas***. Ann. Bat. (N.S.) **19**: 351-371.

Wang, M.C. and N.T. Keen. 1970. Purification and characterization of endopolygalacturonase from ***Verticillium alba-atrum***. Archives of Biochemistry and Biophysis **141**: 749-757.

Weber, K. and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. J. Biol. Chem. **244**: 4406-4412.

Williams, P.H. and N.T. Keen. 1967. Relation of cell permeability alteration to water congestion in cucumber angular leaf spot. Phytopathology **57**: 1378-1385.

Yoshikawa, M., T. Tsukadaira, H. Masago and Minoura. 1977. A nonpectolytic protein from ***phytophthora capsici*** that macerates plant tissues. Physiological plant pathology **11**: 61-70.

從酵素觀點探討病原菌與寄主間之相互關係

I. 探討由 *phytophthora capsici* 產生之聚合半乳醣酸反脫去性內分解酵素及其對植物組織之影響

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從被 *Phytophthora capsici* 感染之茄子果實萃取液中，發現含有對熱穩定之聚合半乳醣酸反脫去性內分解酵素，這種酵素最適合之酸鹼度爲 8.5，需要鈣離子爲輔因子，而且最佳受質爲聚合半乳醣酸。循由硫酸胺鹽析法，DEAE 色層分析法及等電點集中法，我們從病組織萃取液中，將此酵素純化爲600倍。該酵素之分子量估計爲 96,000，等電點爲8.9。再以這些經過純化而不再混雜其他蛋白分解酵素，磷脂分解酵素，聚合半乳醣分解酵素，果膠質甲基反脫去性分解酵素及半纖維素分解酵素之聚合半乳醣酸反脫去性分解酵素，對其與植物組織之影響作進一步的研究，結果發現將茄子組織薄片以此種純化酵素處理30分鐘之後，並未測得組織軟化和死亡之現象，但經過60分鐘後，組織開始急速的軟化，並發現細胞死亡現象，到120分鐘時，組織完全潰爛。將此酵素處理馬鈴薯組織時亦獲相似的結果。令人驚訝的是，這種純化酵素未能導致細胞內電解質之流失，因爲我們發現它跟緩衝液之電導度與控制組相較，並無顯著差異。對於 *P. capsici* 產生之聚合半乳醣酸反脫去性內分解酵素無法導致植物組織改變其滲透性之現象，本文尙未能加以適當的闡釋。