Multiple Forms of Endo-Pectate Lyase Formed in Culture and in Infected Squash Hypocotyls by Hypomyces solani f. sp. cucurbitae

J. G. Hancock

Associate Professor, Department of Plant Pathology, University of California, Berkeley 94720. The technical assistance of Michal Alexander and Colleen Eldridge is gratefully acknowledged.

Accepted for publication 10 July 1975.

ABSTRACT

Differences in several physical properties were found between endo-pectate lyase produced by *Hypomyces solani* f. sp. *cucurbitae* in culture and that produced in *Hypomyces*-infected squash *(Cucurbita maxima).* Endo-pectate lyase from culture and infected tissue possessed isoelectric points of 10.2-10.3 and 10.5-10.6, respectively, and the heat stability of purified endo-pectate lyase from culture was much greater than that of similar preparations from infected tissue. These differences, together with disparities in catalytic properties, indicate that enzymic activities of endo-pectate lyase from culture and infected tissue represent multiple molecular forms. Dichotomy in forms of endo-pectate lyase produced by *H. solani* f. sp. *cucurbitae* in culture and during pathogenesis may reflect either fungal adaptation to different environments or postsynthetic modification of the enzyme.

Phytopathology 66:40-45

While pectate lyase (PAL), pectin lyase (PL) and polygalacturonase (PG) were produced in culture by *Hypomyces solani* f. sp. *cucurbitae*, PAL was the only pectolytic enzyme found in squash hypocotyl lesions caused by this fungus (13). Previous tests indicated that endo-PAL (4.2.2.1) was formed in lesions by *H. solani*, whereas assay procedures suggested that both exo-PAL (4.2.2.2) and endo-PAL were produced in autoclaved squash hypocotyls (13).

The lack of qualitative similarity between pectolytic enzyme systems formed in culture and in diseased tissues by individual pathogenic fungi is now considered common (5). However, in spite of the regularity of these findings, there is some disagreement concerning the distinctness of pectolytic enzymes with different modes of action produced by the same fungus under different conditions (18). Swinburne and Corden (18) provided evidence that the resolution of different pectolytic enzyme entities during purification may reflect inadequacies in extraction and purification techniques rather than the presence of separate enzymes. They suggested that some of the variability encountered between enzymes produced during saprogenesis and during pathogenesis could be brought about by changes in the configuration of enzyme molecules during purification.

Kinetic studies of pectolytic enzyme production in culture have shown a sequential induction of pectolytic enzymes with different modes of action and substrate specificity (1, 9, 12). These results suggest strongly that the production of these different pectolytic activities is governed by different genetic regulatory systems. Nevertheless, alterations in enzyme molecular forms and other artifact problems need to be considered during all purification processes.

Induction processes that regulate the formation of pectolytic enzymes during pathogenesis have been studied in culture because of experimental simplicity (14, 15). However, with the prospect that individual microorganisms produce multiple forms of specific types of pectolytic enzymes, investigators should be cautious before selecting model induction systems. For example, if variant forms represent isozymes; i.e., different proteins with distinct genetic origins that catalyze the same reaction, conditions responsible for isozyme induction in culture may differ significantly from those which regulate production during pathogenesis.

In this study pectate lyase produced in culture and in squash hypocotyls by *H. solani* was purified by several procedures, the properties of the enzymes from the two sources were compared, and biological implications of the results were considered.

MATERIALS AND METHODS.—Stock cultures and inocula of *Hypomyces (Fusarium) solani* f. sp. *cucurbitae* Snyd. and Hans. race 1 were maintained and prepared as described before (13). Only one clone (Cu-56) was used in this work.

Unless stated otherwise, the fungus was cultured on 25 ml of a polygalacturonic acid liquid medium (14) in 125-ml flasks at 25 C. The medium contained 0.05% agar (Ionagar No. 2, Colab Laboratories, Chicago Heights, Ill.). Cultures were not shaken. The liquid was separated from mycelial mats after 5 days of growth, and the culture liquid was filtered through a Millipore filter (pore size 0.45 μm). Filtered liquids were dialyzed against large volumes of water, lyophilized, and stored at −20 C.

Squash (*Cucurbita maxima* Dcne 'Pink Banana') were grown in the greenhouse in U. C. mix (3). Seedlings were inoculated 3-6 days after emergence with 1-ml suspensions of macroconidia (6×10^5/ml) at the base of hypocotyls. Infected stems were harvested 1-3 days after visible lesion development. Lesion areas were removed and used immediately or stored at −20 C.

Enzyme extraction procedures.—Necrotic tissues or healthy hypocotyl tissues were triturated in 0.25 M NaCl for 1 minute in a Waring Blendor and strained through three layers of cheesecloth or one layer of fine-mesh nylon fabric. The filtered materials were centrifuged for 10 minutes at 10,000 *g* and the supernatant liquid dialyzed against water overnight.

Dialyzed materials were used directly in ammonium

sulfate fractional precipitation, lyophilized, or concentrated by ultrafiltration with an Amicon Diaflo membrane apparatus with a PM-10 membrane (Amicon Corp., Lexington, Mass.).

Enzyme assay methods.—Pectate lyase (polygalacturonate *trans*-eliminase) activity was measured by viscosimetric, colorimetric [thiobarbituric acid (TBA)], and direct spectrophotometric procedures identical to those described previously except that the final $CaCl_2$ concentration was 0.1 mM in reaction mixtures used in the direct spectrophotometric method (13). Viscosimetric data were used to calculate relative enzyme units derived from 1,000/t, where t equals minutes required for the relative viscosity of the reaction mixture to be reduced 50%. In the direct spectrophotometric assay, the appearance of Δ4,5-unsaturated bonds during breakdown of pectate by *trans*-elimination was followed at 232 nm with a Cary Model 14 recording spectrophotometer. Protein was estimated in dialyzed extracts by the method of Lowry et al. (16) with crystalline bovine albumin as the standard.

Reaction products were detected by paper chromatography. As a precaution against contaminating sugars, sodium polypectate was cleaned by successive ethanol and diethyl ether washes prior to use. The chromatography solvent consisted of butanol-acetic acid-water (4:3:3, v/v). Duplicate chromatograms were run with standard galacturonic acid and reaction products were localized with the quinine sulfate and aniline reagents (13) or bromphenol blue (17).

Purification techniques.—The first step in purification of PAL from dialyzed tissue extracts was with ammonium sulfate fractional precipitation. The protein fraction that precipitated between 50 and 90% ammonium sulfate saturation contained nearly all of the original PAL activity. Before further use, the precipitate formed at 90% saturation was dialyzed against the equilibrating buffer to be used in column chromatography and assayed for PAL activity. Lyophilized culture precipitations were resuspended in the column buffer prior to use.

Sephadex G-100 (Pharmacia Fine Chemicals, Uppsala, Sweden) was used most commonly in gel filtration. Gels were swollen and columns (3 × 40 cm) were prepared by methods suggested by the manufacturer with the gel suspended in 20 mM Tris · HCl and 0.02% sodium azide. Columns were eluted with buffer at about 0.25 ml/minute at pH values of 6.5, 7.5 or 8.5. Fractions of 5 ml were usually collected at 4 C.

The molecular weight of PAL from culture and infected tissues was estimated by gel filtration using a procedure described by Andrews (2). Blue Dextran (mol. wt. 2,000,000), cytochrome C (mol. wt. 12,500), bovine serum albumin (mol. wt. 67,000), and yeast alcohol dehydrogenase (mol. wt. 150,000) were used as reference standards.

Diethylaminoethyl (DEAE)-cellulose or DEAE-Sephadex (A-50, Pharmacia) anion exchange column chromatography was used to purify PAL from culture and infected tissue. Column materials were equilibrated with 10 mM Tris · maleate (pH 6.5) or Tris · HCl (pH 6.5, 7.5, or 8.5), and 0.05 M NaCl and the samples eluted with a NaCl linear gradient from 0.05 M to 0.25 M NaCl at 4 C. With a second procedure the samples were eluted with the equilibrating buffer (10 mM Tris · HCl) with or without 0.15 M NaCl.

Dialyzed, concentrated preparations from culture filtrates and tissue extracts were directly subjected to isoelectric focusing with pH 3-10 or pH 9-11 Ampholine carrier ampholytes (LKB-Produkter AB, Bromma, Sweden) in a 110-ml electrofocusing column (LKB 8101 electrofocusing apparatus). Samples were electrofocused at 4 C until the current had stabilized at 300 V and 0.7 ma (72 - 96 hours). Two- or 3-ml fractions were collected at the rate of 2 ml/minute. The pH of each fraction was determined immediately at 4 C. Fractions were dialyzed prior to the TBA assay for PAL.

RESULTS.—*Purification.*—A significant degree of purification of PAL from infected tissue or culture required at least two steps with high resolution procedures (Table 1). Used alone, Sephadex G-100 or DEAE-Sephadex (A-50) yielded a 4- to 8-fold purification of PAL from $(NH_4)_2SO_4$ precipitates of extracts of infected tissue or of lyophilized culture filtrates. A second step with one of these procedures increased the overall purification factor to 20- to 30-fold.

TABLE 1. Purification of pectate lyase from culture filtrates of *Hypomyces solani* f. sp. *cucurbitae* and from squash hypocotyls infected by that fungus

Purification step	Specific activity[a] (units/mg protein)	Purification factor
Culture		
Dialyzed, lyophilized	588	1.0
DEAE, pH 8.0	715	1.2
DEAE, pH 6.0	11,579	19.7
Infected Tissue		
Dialyzed	577	1.0
Ammonium sulfate ppt.	909	1.6
DEAE, pH 8.0	2,163	3.8
Sephadex G-100	15,111	26.2

[a]Pectate lyase activity was measured by the viscosimetric method.

Resolution of exo-pectate lyase and endo-pectate lyase.—Elution patterns of PAL from Sephadex G-100 or DEAE-Sephadex columns allowed a comparison of activity profiles using the viscosimetric and *trans*-elimination (spectrophotometric) assay procedures (Fig. 1-4). A small resolution of two PAL activities was noted during elution of culture materials from Sephadex G-100 and DEAE-Sephadex columns (Fig. 1, 3). However, isoelectric focusing of culture materials yielded a substantial resolution of PAL activities where viscosimetric activity focused at pH 10.2 and the largest portion of *trans*-elimination activity focused at pH 8.9 (Fig. 5).

Each of the enzyme purification techniques used in this study showed that PAL activity in culture could be resolved into two enzymic types. In contrast, patterns of PAL activities in eluents from columns loaded with extracts of infected tissue were parallel regardless of the assay method (Fig. 2, 4).

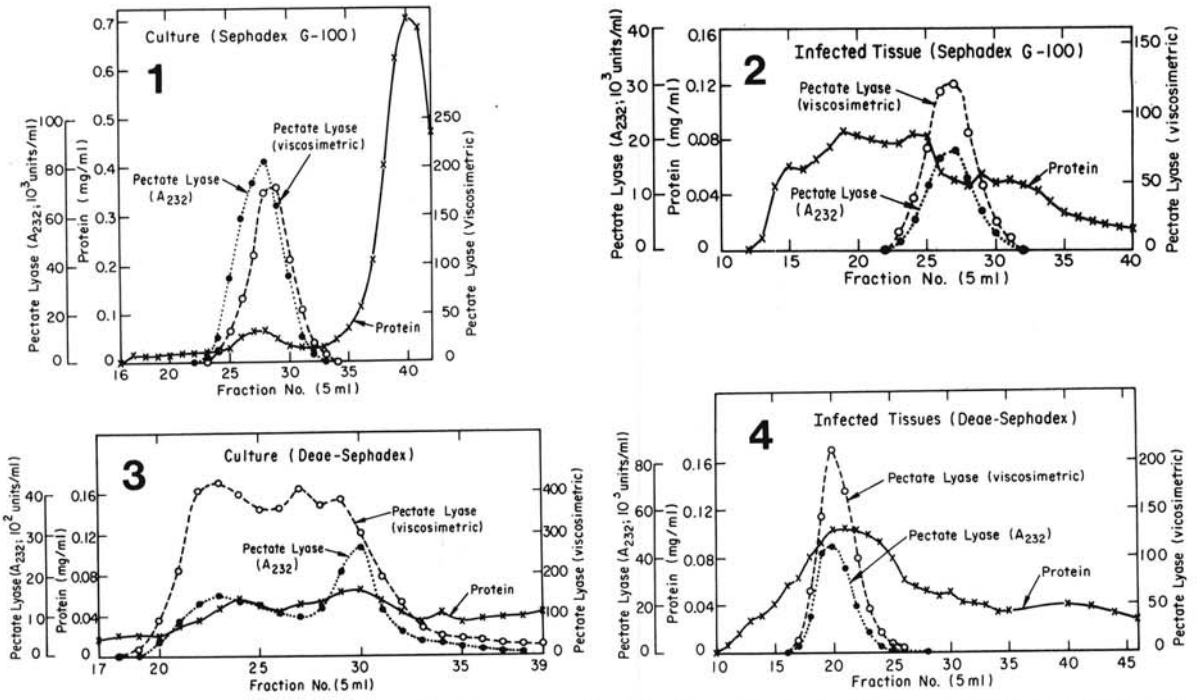

Fig. 1-4. Purification and chromatography of pectate lyase produced in vitro and in vivo by *Hypomyces solani* f. sp. *cucurbitae*. **1)** Gel filtration of pectate lyase in lyophilized culture filtrates on Sephadex G-100, pH 6.0. Pectate lyase was assayed by spectrophotometric (A_{232}) and viscosimetric techniques. **2)** Gel filtration of pectate lyase in an ammonium sulfate precipitate of an extract of *Hypomyces*-infected squash hypocotyls on Sephadex G-100, pH 6.0. **3)** DEAE-Sephadex chromatography of pectate lyase in lyophilized culture filtrates eluted with 0.15 M NaCl (pH 6.5). **4)** DEAE-Sephadex chromatography of pectate lyase in an ammonium sulfate precipitate of an extract of *Hypomyces*-infected squash hypocotyls eluted with 0.15 M NaCl (pH 6.5).

Larger ratios of α-1, 4-glycosyl bond cleavage (*trans*-elimination) to reduction in pectate viscosity were noted when enzyme activities from autoclaved hypocotyl (13) or liquid culture were compared with those from infected tissues. Because successive end-wise release of sugar residues from pectate polymers has less effect on substrate viscosity than internal bond cleavage, these results suggested that cultures contain higher levels of exo-PAL than infected tissues. Purification work in this study indicated that exo-PAL is present in liquid culture but not in infected tissues. Similar differences were observed between activities of PAL produced by *Colletotrichum trifolii* in culture and in infected alfalfa stems (J. G. Hancock, *unpublished*).

Significant pectate viscosity reducing activity accompanied by a low degree of depolymerization can be attributed to random cleavage of internal glycosidic bonds (5). In previous work it was noted that only about 0.5% cleavage of glycosidic bonds was required to cause a 50% reduction in viscosity of sodium polypectate by PAL from infected tissue (13). In this study it was consistently found that about 1% of the bonds were cleaved by purified PAL from culture in causing a 50% reduction in substrate viscosity. In spite of the differences between enzymes from the two sources, the small amount of bond cleavage in relation to viscosity loss suggests that both enzymes should be classified as endo-PAL type pectolytic enzymes. Except in the presence of exo-PAL, paper chromatography of reaction mixtures after pectate breakdown showed that endo-PAL from both sources yielded identical oligosaccharides, all of which possessed absorption maxima at 232 nm, evidence for the presence of Δ4,5 unsaturated bonds.

Endo-PAL from infected tissues eluted as a single peak from Sephadex G-100 and DEAE-Sephadex columns (Fig. 2, 4). This elution pattern persisted with DEAE ion exchange chromatography regardless of whether the column was eluted with or without a concentration gradient of NaCl.

Single peaks were noted when endo-PAL from culture was purified on Sephadex G-100 with buffers of pH 6.5 whereas two broad peaks were observed when DEAE-Sephadex columns were eluted with 0.15 M NaCl at pH 6.5 (Fig. 3). Upon rechromatography under the original conditions, pooled fractions from each endo-PAL peak from DEAE-Sephadex emerged as a single peak and according to its original position. If DEAE-Sephadex columns were eluted with NaCl concentration gradients between 0 and 50 mM, endo-PAL eluted as an initial sharp, high peak (I), followed after 18-5 ml fractions by a broad,flat peak (II).

Properties of endo-pectate lyase from culture and infected tissue.—Molecular weights of endo-PAL from culture and infected tissue were estimated by gel filtration on Sephadex G-100, using enzymes with known molecular weights as standards (2). Relative elution patterns of endo-PAL and standards indicated that endo-PAL from culture and infected tissue possess molecular

sizes between 32 and 42 × 10^3 daltons. Although differences were not distinct enough to draw conclusions, comparisons of elution patterns suggested that endo-PAL from infected tissue was slightly larger than endo-PAL from culture.

With the isoelectric focusing technique, single endo-PAL peaks were found during purification of either dialyzed extracts of infected tissue or dialyzed culture filtrates (Fig. 5, 6:insert). Using a narrow range ampholyte (pH 9-11), isoelectric points (pI) of the peaks of endo-PAL from the two sources were established. The peak of activity from culture possessed a pI of 10.2-10.3 whereas the peak from infected tissue had a pI of 10.5-10.6. The pI values of the enzymes from the two sources were consistent during the course of this study. When dialyzed culture filtrates and tissue extracts were mixed and electrofocused, two endo-PAL peaks were resolved

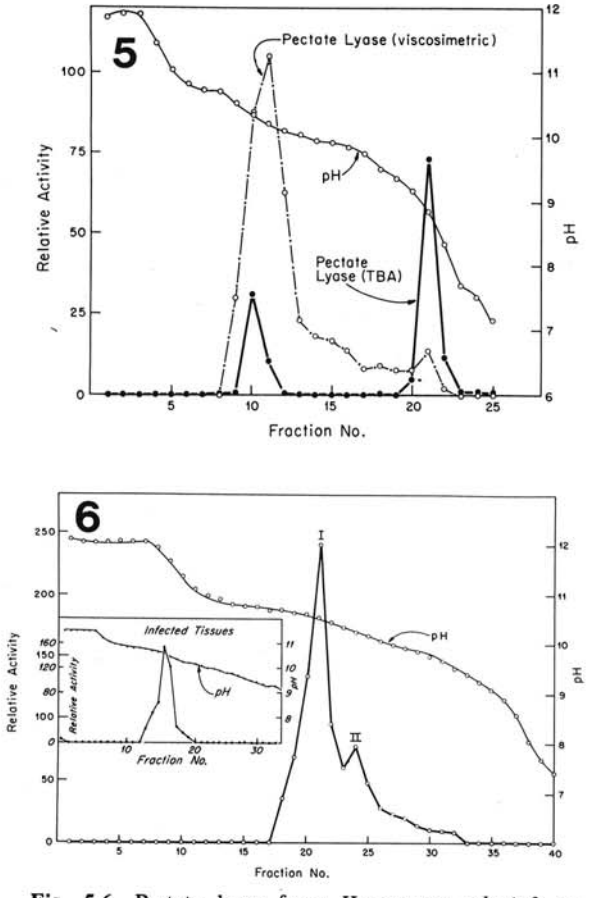

Fig. 5-6. Pectate lyase from *Hypomyces solani* f. sp. *cucurbitae*. **5)** Electrofocusing of pectate lyase in culture filtrates performed with pH 9-11 ampholytes for 96 hours at 4 C. Pectate lyase was assayed by the thiobarbituric acid (TBA) and viscosimetric procedures. **6)** Electrofocusing of pectate lyase in a mixture of dialyzed culture filtrates and extracts of *Hypomyces*-infected squash hypocotyl tissues. Activities of pectate lyase in peaks I and II were, respectively, completely and partially (20% of original activity) inactivated after 20 minutes at 50 C. *Insert:* Electrofocusing of pectate lyase from *Hypomyces*-infected squash hypocotyl tissues. Pectate lyase was assayed by the viscosimetric procedure.

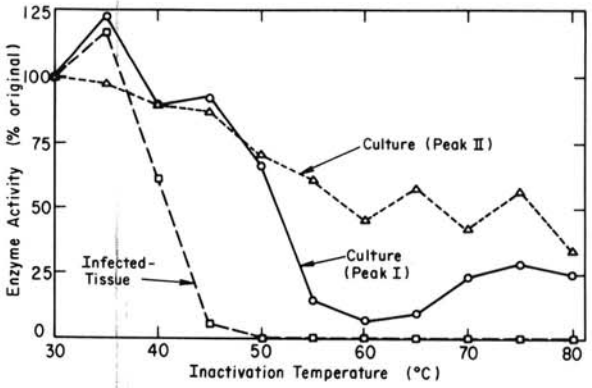

Fig. 7. Relative activities of endo-pectate lyase from *Hypomyces solani* f. sp. *cucurbitae*-infected tissues and culture filtrates after 20 minutes at designated temperatures. All preparations were partially purified on DEAE-Sephadex prior to heat treatment. Culture preparations were from an early, sharp peak (I) and a late, broad peak (II) after gradient elution (0-0.05 M NaCl).

(Fig. 6). Peak I, with a pI of 10.5, was totally destroyed when fractions from the peak were heated to 50 C for 20 minutes. Fractions from peak II (pI 10.2) retained 20% of their original activity after heat treatment. Since endo-PAL from culture is more heat stable at 50 C than endo-PAL from infected tissues (Fig. 7), these results support the notion that peak I is the enzyme from infected tissues and that peak II contains activity from culture and that these activities possess different isoelectric points.

Thermal inactivation of partially purified endo-PAL from infected tissues was complete after treatment at 50 C for 20 minutes or 55 C for 10 minutes. However, after heating dialyzed filtrates from liquid culture or extracts of squash hypocotyl culture at 50 C for 20 minutes, about 60% of the original endo-PAL activity was retained. About 5% of the original activity remained after heating at 100 C for 20 minutes. The heat stability of endo-PAL from liquid culture was similar after purification by either gel filtration, ion exchange chromatography, or isoelectric focusing.

The two main endo-PAL peaks that resulted from linear gradient elution of culture materials from DEAE-Sephadex with NaCl (0 - 50 mM) had high heat stabilities (Fig. 7). Endo-PAL activity in peak II was more heat stable than that in peak I. The pattern of the peak I heat inactivation curve was similar in several different experiments and was reminiscent of patterns found with other pectolytic enzymes (10).

The influence of several monovalent and divalent cations (0.1 mM) were tested on the activities of endo-PAL from culture and infected tissue. Relative viscosimetric activities of partially purified enzymes from both sources were adjusted to identical levels. Under these conditions it was found that Mg^{++}, Na^{+}, and K^{+} had little effect on endo-PAL from either source, whereas Ca^{++} stimulated the activity of enzymes from both sources about 50%. Ba^{++} inhibited endo-PAL from culture and infected tissue 75% and 45%, respectively. Although Li^{++} inhibited the enzyme from culture 30%, it did not influence endo-PAL from infected tissue appreciably.

The pH optima of endo-PAL from culture and infected tissue was about 9.5, with the enzyme from infected tissue possessing a broader activity profile than the one from culture. Endo-PAL from infected tissue maintained 25% and 45% of its optimum activity at pH 7.5 and 8.0, whereas the enzyme from culture yielded 5% and 15% of its optimum at these pH values.

Relative endo-PAL activity between pH 7.5 and 8.0 could be of biological importance since the pH of hypocotyl tissues is raised to within this range after infection by *H. solani* (13). It appears that endo-PAL produced in infected tissue would be relatively more effective during pathogenesis in degrading host pectic substances than the enzyme produced in culture.

Enzyme activity in healthy tissue.—Low levels of endo-PAL were noted consistently in extracts from healthy squash hypocotyls. With the viscosimetric assay, enzyme levels in healthy hypocotyls averaged about 5 units/g fresh weight compared to around 1,500 units/g fresh weight in lesions. High levels of activity were only present in necrotic tissue while living host tissues bordering lesions contained activities similar to those in healthy hypocotyls. Unfortunately, it has not been possible to compare the physical properties of the endo-PAL from healthy tissue with the one from necrotic tissue because of the former's low activity and lability during purification. Although it is possible that the endo-PAL in necrotic tissue is of host origin, it appears unlikely given the close association between sites of fungal growth and high endo-PAL activity. Nevertheless, this possibility appears worthy of further investigation, particularly given that host pectolytic enzymes can increase after infection (19).

DISCUSSION.—Results suggest important differences in properties exist between endo-PAL from culture and infected tissues. Consistent discrepancies in isoelectric points, striking differences in heat stabilities, and disparities in catalytic properties strongly imply that enzymic activities of endo-PAL from the two sources represent multiple forms. Nevertheless, more critical studies on the chemistry and configuration of these two forms are required before it can be concluded that they are isozymes.

Endo-PAL from *Hypomyces*-infected squash hypocotyls is composed of a single form while there appear to be two endo-PAL components produced in culture. However, the pattern of resolution of endo-PAL activities in culture fluids by isoelectric focusing and ion exchange chromatography suggests that cultures contain a single electrolytically homogeneous enzyme and a fraction of activity that possesses a mixed charge. The flat, broad peak resulting from gradient elution of DEAE exchange columns and the "tailing" of endo-PAL activity after electrofocusing may represent an altered form of the enzyme found in the sharply defined peaks in the same culture materials.

Differences in properties of endo-PAL could result from postsynthetic modification during purification. Since properties of endo-PAL from culture were similar after purification by different procedures, alterations in properties are not likely to have occurred during this process. While there is no direct evidence in this regard in this study, enzyme modification could take place after enzyme secretion into culture fluids. Further work on this question is required, however, before conclusions can be drawn on the origin of multiple forms of endo-PAL produced by *H. solani* f. sp. *cucurbitae*.

Plant pathogenic microorganisms are capable of producing multiple forms of individual types of pectolytic enzymes in culture (7, 11, 20) and there is now evidence for the production of separate forms in culture and in infected tissues. For example, Brookhouser (6) recently reported that *Rhizoctonia solani* produces two forms of endo-PG (3.2.1.15) in infected cotton hypocotyls, only one of which is found in culture. This work supports Bateman's (4) earlier observation that there are major differences in the endo-PG types produced by *R. solani* in culture and in infected bean hypocotyls. With another host-parasite system, published data indicates that *Sclerotinia fructigena* produces a form of endo-PG in infected apples not found in culture or healthy apples (8). Formation of endo-PAL by *H. solani* in culture and infected tissues differs from these results only in that pectolytic activity in infected tissue is accounted for by a single form of endo-PAL.

Production of multiple forms of endo-PAL in culture and in infected tissue was consistent and appeared to be strictly related to the conditions under which *H. solani* was grown. Yet preliminary experiments indicated that PAL systems produced in autoclaved squash hypocotyls were identical to those produced in liquid culture, suggesting that the types of pectolytic enzymes *produced in culture* may be formed over a range of growth conditions. An investigation of the influence of a wide variety of cultural conditions on the production of multiple forms of endo-PAL by *Hypomyces* is needed. This work could provide insight into the relationship between enzyme induction processes and the specialized parasitic behavior of pathogenic microorganisms.

Investigators should be greatly concerned with variant forms of individual types of pectolytic enzymes produced by microorganisms in culture and during pathogenesis. Regardless of their origin (i.e., artifacts or isozymes), if enzymes studied from culture are composed of multiple forms distinct from those produced during pathogenesis, evaluations of their biological properties (e.g., host cell-killing capacity, macerating ability) may be inaccurate from the view of disease.

LITERATURE CITED

1. ALBERSHEIM, P., T. M. JONES, and P. D. ENGLISH. 1969. Biochemistry of the cell wall in relation to infective processes. Annu. Rev. Phytopathol. 7:171-194.
2. ANDREWS, P. 1964. Estimation of the molecular weights of proteins by Sephadex gel-filtration. Biochem. J. 91:222-233.
3. BAKER, K. F., ed. 1957. The U.C. system for producing healthy container-grown plants. Calif. Agric. Exp. Stn. Man. No. 23. 332 p.
4. BATEMAN, D. F. 1963. Pectolytic activities of culture filtrates of Rhizoctonia solani and extracts of Rhizoctonia-infected tissues of bean. Phytopathology 53:197-204.
5. BATEMAN, D. F., and R. L. MILLAR. 1966. Pectic enzymes in tissue degradation. Annu. Rev. Phytopathol. 4:119-146.
6. BROOKHOUSER, L. W. 1974. Characterization of endo-polygalacturonase produced by Rhizoctonia solani during infection of cotton seedlings and in response to host exudates. Ph.D. Thesis, University of California, Berkeley. 62 p.

7. BYRDE, R. J. W., and A. H. FIELDING. 1968. Pectin methyl-transeliminase as the maceration factor of Sclerotinia fructigena and its significance in brown rot of apple. J. Gen. Microbiol. 52:287-297.
8. BYRDE, R. J. W., A. H. FIELDING, S. A. ARCHER, and E. DAVIES. 1973. The role of extracellular enzymes in the rotting of fruit tissue by Sclerotinia fructigena. Pages 39-54 *in* R. J. W. Byrde and C. V. Cutting, eds. Fungal pathogenicity and the plant's response. Third Long Ashton Symposium, 1971. Academic Press, N.Y.
9. COOPER, R. M., and R. K. S. WOOD. 1975. Regulation of synthesis of cell wall degrading enzymes by Verticillium albo-atrum and Fusarium oxysporum f. sp. lycopersici. Physiol. Plant Pathol. 5:135-156.
10. FIELDING, A. H., and R. J. W. BYRDE. 1969. The partial purification and properties of endopolygalacturonase and α-L-arabinofuranosidase secreted by Sclerotinia fructigena. J. Gen. Microbiol. 58:73-84.
11. GARIBALDI, A., and D. F. BATEMAN. 1971. Pectic enzymes produced by Erwinia chrysanthemi and their effects on plant tissue. Physiol. Plant Pathol. 1:25-40.
12. HANCOCK, J. G. 1966. Pectate lyase production by Colletotrichum trifolii in relation to changes in pH. Phytopathology 56:1112-1113.
13. HANCOCK, J. G. 1968. Degradation of pectic substances during pathogenesis by Fusarium solani f. sp. cucurbitae. Phytopathology 58:62-69.
14. HANCOCK, J. G., C. ELDRIDGE, and M. ALEXANDER. 1970. Characteristics of pectate lyase formation by Hypomyces solani f. sp. cucurbitae. Can. J. Microbiol. 16:69-74.
15. HORTON, J. C., and N. T. KEEN. 1966. Sugar repression of endopolygalacturonase and cellulase synthesis during pathogenesis by Pyrenochaeta terrestris as a resistance mechanism in onion pink root. Phytopathology 56:908-916.
16. 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.
17. PAGE, O. T. 1961. Quantitative paper chromatographic techniques for the assay of products of polygalacturonase activity of fungus cultures. Phytopathology 51:337-338.
18. SWINBURNE, T. R., and M. E. CORDEN. 1969. A comparison of the polygalacturonases produced in vivo and in vitro by Penicillium expansum Thom. J. Gen. Microbiol. 55:75-87.
19. TANI, T. 1967. The relation of soft rot caused by pathogenic fungi to pectic enzyme production by the host. Pages 40-57 *in* C. J. Mirocha and I. Uritani, eds. The dynamic role of molecular constituents in plant-parasite interaction. American Phytopathological Society, St. Paul, Minn.
20. TANI, T., and H. NAMBA. 1969. Qualitative nature of macerating activities in the culture filtrates of Botrytis cinerea. Ann. Phytopathol. Soc. Jap. 35:1-9.