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SUMMARY OF RESULTS OF STUDIES COMPLETED ON THE PRIOR

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"Role of Proteolytic Enzymes in Degradation of Plant Tissues"

J. Lewosz, A. Kelman, and L. Sequeira

Strain SR 394 of Erwinia carotovora (Ecc) produced proteases constitutively in all media tested. Growth of Ecc and production of protease were enhanced significantly by the presence of pectic materials and/or plant cell walls in the test media. After electrofocusing, one major and one minor protease bands, at PI 4.8 and PI 5.1, respectively, were detected. Only one band of 43 kDa was detected on SDS gels. Only one protease band was detected in SDS gels of infected plant extracts. This protease was purified to homogeneity. It is a highly thermostable metal protease; it degrades gelatin, soluble collagen and hide powder azure, shows weak activity on casein and azocasein, but does not degrade insoluble collagen or elastin.

Bacteria grown on modified polypectate medium containing 0.5% hydroxyproline-rich glycoproteins (HPRGs) from either carrot root or potato tubers did not produce additional proteases, as compared with unmodified media.

Crude Ecc protease preparations caused rapid release of electrolytes from potato tuber slices, but this was due to the presence of pectic lyase (PL) activity in the extracts. Inhibition of proteolytic activity by alpha-macroglobulin did not affect electrolyte release by crude culture filtrates. Purified Ecc protease did not bind to plant cell walls, whereas PL bound very rapidly.

Approximately 20% of the total hydroxyproline content of cell walls was liberated by incubation with bacterial filtrates containing both protease and PL activities. Inactivation of PL by heating for 2 min at 60 C stopped the release of hydroxyproline-containing materials, even though more than 60% of the initial proteolytic activity was still present. The amount of hydroxyproline materials released by crude filtrates was the same in the presence or absence of the protease inhibitor, alpha-macroglobulin. This indicated that PL was essential for the liberation of hydroxyproline-containing materials from cell walls along with pectic oligomers.

It is evident that extensin-like proteins in cell walls are not readily accessible to proteolytic degradation. Degradation of extensin required the synergistic action of protease and PL from Ecc. When about 50% of the HPRG in cell walls was solubilized by boiling at pH 1.0, only a portion of the HPRGs remaining in the cell walls was released after prolonged incubation with Ecc culture filtrates. The solubilized material was degraded only slightly after incubation with purified Ecc protease. Similarly, HPRGs isolated from wounded carrot roots were not degraded by the Ecc protease. However, these preparations contained oligomeric, cross-linked HPRGs that had a tendency to precipitate out of solution.

Since growth of Ecc in infected tissues results in reducing conditions, there was a possibility that such conditions are necessary for degradation of soluble HPRGs, since cross-linking would be inhibited. When 5 mM mercaptoethanol was used to prevent cross-linking of extensins isolated from tobacco cell suspension cultures, presumably by inhibiting peroxidase activity, HPRGs were degraded by Ecc protease. These extensin precursors were cleaved into peptides of different sizes, but not to free hydroxyproline. When the same HPRG preparations were cross linked by adding peroxidase and H_2O_2, this oligomeric fraction was not degraded by Ecc protease.

Publications.-

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