

Title Purification and characterization of polygalacturonase inhibiting proteins (PGIP) from two varieties of Asian pear

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Abstract

PGIPs are plant proteins that inhibit fungal polygalacturonases (poly (1, 4-D galacturonide) glycanohydrolases ; EC 3.2.1.15). PGIPs are thought to contribute to plant defense responses against pathogens. Plant material, Asian pears cvs. Shinli and Shinko were frozen in liquid N₂ and used immediately or stored at -20°C until use. PG sources from *Aspergillus niger* commercial pectinase (Sigma Aldrich, USA), *Botrytis cinerea* and *Colletotrichum acutatum* were used as the sources of PGs. PGIP extraction 30 gr of Shinli and Shinko pear fruits were processed. Ex-tracts were dialyzed overnight against buffer, and then used to assay PGIP activity. Inhibition of endo-PG activity from *A. niger* commercial pectinase (Sigma Aldrich, USA), *C. acutatum* and *B. cinerea* was assayed, using a gel diffusion assay according to Taylor and Secor, (1988). For PGIP purification fruit were homogenized in an equal volume of extraction buffer, and applied to a column of Con A-Sepharose 48. Chromatography was performed at 0.5 ml/min. Protein bound to the column was eluted using 750 mM methyl a D-manno pyranoside in Con A buffer. The eluent was dialyzed against 50 mM sodium acetate, pH 4.5 (buffer A), and then concentrated by ultrafiltration using a pressure cell fitted with a PM-10 membrane (Amicon, Danvers, MA). Asian pear cultivars Shinli and Shinko showed different PGIP inhibition against different PGs from distinct organisms; PGIP inhibition activity was strictly pH dependent against *A. niger*. at pH 5.75; it was not significantly affected by ionic strength; but susceptible to temperature, and reduced by 80-90% at 80°C for 10 minutes. It was neither susceptible to extracting time, nor to NaCl content in extracting buffer. Its activity from different tissues was completely different (fruits < spurs > flowers > leaves). It was a sugar bound protein. However, it was fractioned by SEC at its size 4.8×10^5 , but it was not sensitive to IEC.