

Title Isolation, purification, and characterization of a polygalacturonase produced in *Penicillium solitum*-decayed 'Golden Delicious' apple fruit

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Abstract

Polygalacturonase (PG) was extracted and purified from decayed 'Golden Delicious' apple fruit inoculated with *Penicillium solitum*. Ammonium sulfate, gel filtration, and cation exchange chromatography were used to purify the enzyme. Both chromatographic methods revealed a single peak corresponding to PG activity. The purified PG most likely originates from the fungus because PG activity from healthy and wounded apple tissue was undetectable. Analysis of cation exchange-purified material using sodium dodecyl sulfate polyacrylamide gel electrophoresis revealed a single 50-kDa band. The enzyme was active over a broad pH range (3 to 7), with optimal activity between pH 4 and 5. PG was highly active at 20 and 37°C but was also detectable at 2, 50, and 75°C. Divalent cations affected PG enzyme activity; Mg and Fe increased, whereas Ca and Mn reduced activity in vitro. Thin-layer chromatographic separation of hydrolysis products and data from a PG plate activity assay based on staining with ruthenium red showed that the enzyme exhibits both exo and endo activity. Purified PG incubated with intact apple fruit tissue in vitro caused a 30% reduction in mass after 48 h, suggesting a role in *P. solitum*-mediated decay of apple fruit.