Abstract

Rhamnogalacturonan hydrolase, also known as RG-hydrolase (RGase A), cleaves α-1→2 linkages between non-esterified galacturonosyl and rhamnosyl residues of the pectic backbone. RGase A is suspected to be involved in pathogenesis of *Botrytis cinerea*, an important postharvest pathogen, because of its ability to degrade the highly branched pectic backbone.

The aims of this study were to isolate and characterize the *B. cinerea* RGase A gene. The initial approach was to purify RGase A from *B. cinerea*. A new assay method, using α -rhamnosidase, was developed to assay for RGase A activity in column fractions during purification. Using the α -rhamnosidase assay method, a 60 kDa RGase A was purified from crude *B. cinerea* extract by anion exchange, cation exchange and gel filtration. Because of a low yield of pure RGase A protein, an alternate approach was taken.

Isolation of a cDNA clone for the *B. cinerea* RGase A gene was achieved using a published nucleotide sequence for the *Aspergillus aculeatus* RGase A gene transcript. Three gene-specific primers were designed and used to synthesize the RGase A cDNA from *A. aculeatus*. A 1100 bp PCR fragment was obtained using these three primers. Using the PCR-amplified *A. aculeatus* RGase A clone as a probe, a 1.9 kb RGase A cDNA clone (BCRHGA) was isolated from a *B. cinerea* cDNA library.

The coding region of the BCRHGA clone shares 62% identity at the amino acid level with *A. aculeatus* RGase A. Northern blot analysis using *B. cinerea* total RNA revealed a 2 kb band when the BCRHGA cDNA was used as a probe, showing that the BCRHGA clone is a nearly full length cDNA clone. To determine the expression profile of the RGase A gene, *B. cinerea* spores were grown in Richmond media containing either 0.5% apple pectin, 0.5% rhamnogalacturonan-I (RG-I) or 1% glucose as a carbon source. Northern blot analyses revealed that the *B. cinerea* RGase A gene was expressed in cells grown on all three carbon sources. With 0.5% apple pectin and RG-I, maximum expression was reached in three-day-old cells and then dropped after day three. A different gene expression pattern was obtained when cells were grown on 1% glucose, in which RGase A expression increased progressively from day one to six. *B. cinerea* RGase A appeared to be coded for by a single

or low copy number gene based on Southern blot analysis. Finally, the *B. cinerea* RGase A clone has been expressed in Escherichia coli, for use to obtain sufficient protein for antibody production.