Purification of enzyme and influence of substrate specificity on â-1,6-glucanase gene expression in Trichoderma harzianum

ABSTRACT

ß-1,6-glucanase produced by Trichoderma harzianum has been proven as one of the prime compounds to be excreted onto the hyphae of the pathogen causing localised cell wall lysis at the point of interaction. This study was conducted in the interest to investigate the regulation of ß-1,6-glucanase gene expression in T. harzianum strain BIO10671. β-1,6-glucanase enzyme from the culture filtrate of T. harzianum was purified through precipitation with 80% acetone followed by anion-exchange chromatography and chromatofocusing using Neobar AQ and Mono P HR 5/20 columns, respectively. Two β-1,6-glucanase bands at 32 kDa and 43 kDa in size has been purified. However, four restriction endonucleases digestion revealed only a single copy of ß-1,6-glucanase gene was encoded for both ß-1,6-glucanase isozymes. Fungal cell walls were able to trigger high level expression of gene encoding ß-1,6-glucanase. The expression of ß-1,6-glucanase gene was strongly affected by substrate specificity; where the presence of glucose or non ß-1,6-glucan linked substrate will significantly suppress the gene transcriptions. In spite of this, 24 hours were required for the gene transcription to achieve maximum total mRNA.

Keyword: ß-1,6-glucanase; Gene; mRNA; Purification; Substrate specificity