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# Characterization of the endo-1,4-D-xylanase (EXN) gene of Trichoderma virens with a view to develop a hemicellulose utilizing recombinant yeast system

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Xylanases, including *endo*-1,4-D-xylanase are fundamental enzymes that convert hemicellulose biomass to fermentable sugars. Many filamentous fungi are known to secrete high amounts of *endo*-1,4-D-xylanase enzyme. A part of our ongoing study included the development a recombinant *Pichia stipitis* which is proficient in converting hemicellulose biomass to fermentable sugars efficiently. This required the cloning of the *endo*-1,4-D-xylanase (EXN) gene. The filamentous fungi *Trichoderma virens* which is an efficient secretor of *endo*-1,4-D-xylanase was chosen for isolating the gene for this enzyme.

Forward and reverse primers (EXNFP and EXNRP) were designed and the polymerase chain reaction was carried out to amplify the EXN gene using genomic DNA of *T. virens* as template. A product of expected size was obtained and ligated with pGEM-T easy vector system II and transformed in *E. coli* JM109 cells. Recombinant plasmids were isolated and sequenced.

The size of the gene was 690 bp (without signal peptide) with a single intron (115 bp) from 274 to 387 bp. The coding region contained a 573 bp open reading frame encoding 191 amino acids. Sequence analysis of the mature protein revealed 100% similarity to the amino acid sequence of *endo*-1,4-beta-xylanase of *Trichoderma viride* (AAP83925.1). The amino acid sequence was identified as belonging to glycosyl hydrolases family 11 where most of the xylanases are included. The characterized gene will be cloned in frame with the α factor signal sequence of pGAPZαA vector for expression in the *Pichia stipitis* system.

Keywords: Endo-1,4-D-xylanase, fermentable sugars, filamentous fungi, glycosyl hydrolases, hemicelluloses

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