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Cloning and characterization of 1,4-β-D-glucosidase (BGL) gene from *Trichoderma* with a view to develop a recombinant yeast system for the conversion of cellulose to ethanol

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Second generation bio-ethanol production has become a key area of research and development. Cellulose in plant biomass is the major target for production of ethanol through hydrolysation using cellullase enzymes. Complete degradation of cellulose involves synergistic action of 3 enzymes namely, endo-1,4-β-D-glucanase (EG), cellobiohydrolase (CBH) and 1,4-β-D-glucosidase (BGL). Among all microbial genera *Trichoderma* species are known to be good cellulase producers and has been widely studied. The long term objective of this study was to clone the cellulase genes from *Trichoderma* into yeast to develop a recombinant yeast system harbouring cellulase, capable of producing ethanol from ligno-cellulosic biomass.

Genomic DNA was extracted from locally isolated *Trichoderma* species which were morphologically similar to *Trichoderma viride*. Primers were designed using available sequences of *T. viride* retrieved from NCBI for PCR amplification of the 3 genes. The 1,4-β-D-glucosidase (BGL) gene was successfully PCR amplified, cloned and characterized. The size of the gene was 1439 bp with one intron (76 bp) from 41 bp to 117 bp. It was 90 % similar to the DNA sequence and 99% similar to the deduced amino acid sequence of 1,4-β-D-glucosidase of *T. atroviride* (AC237343.1). A search of the conserved domain database at NCBI with the deduced amino acid sequence of BGL indicated that it belongs to the glycosyl hydrolases family 1.Subsequent molecular identification of the locally isolated *Trichoderma* species using internal transcribed spacer (ITS) confirmed it to be 100 % homologous to *T. virens* (KJ739790.1). The successful amplification of the BGL gene using *T. viride* specific PCR primers were possible because the BGL genes in *Trichoderma* genus were conserved with little genetic variation compared to the other two genes (EG & CBH). The genetic diversity reported for EG and CBH genes were high differentiating these genes into several types within the *Trichoderma* genus and thus the two genes, EG and CBH did not amplify when *T. viride specific primers* were used for PCR.

Keywords: 1,4-β-D-glucosidase, cellobiohydrolase, endo-1,4-β-D-glucanase, glycosyl hydrolases, second generation bio-ethanol

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