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Cloning and expression of 1,4-β-D-glucosidase, endo-β-D-glucanase and cellobiohydrolase genes of *Trichoderma* in yeast for the second generation bio-ethanol production from ligno-cellulosic biomass

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Ligno-cellulosic biomass comprising of cellulose, hemicellulose and lignin is the most abundant renewable organic resource on earth and it has been the main target for enzyme based second generation bio-fuel production. Complete degradation of cellulose into glucose involves simultaneous action of the three cellulase enzymes, namely endoglucanase (*EGLI*), cellobiohydrolase (*CBHI*) and β-glucosidase (*BGLI*). Among all microbial genera, filamentous fungi *Trichoderma* species are known to be excellent protein secretors including cellulase. This study was focused on cloning three cellulase genes from *Trichoderma* into *Saccharomyces cerevisiae* to develop a recombinant yeast system, harbouring cellulase capable of producing ethanol from ligno-cellulosic biomass. Locally isolated *Trichoderma virens* species expressing cellulase was used to characterize and isolate the genes. The 1,4-β-D-glucosidase I (*BGLI*) gene was successfully PCR amplified using genomic DNA. Endo-1,4-β-D-glucanase I (*EGLI*) and cellobiohydrolase I (*CBHI*) genes of *Trichoderma* were custom synthesized and all three genes were separately cloned into pGAPZα vector. They were individually transformed into *S. cerevisiae* and the recombinant clones were confirmed by colony PCR and sequencing. The expression of cloned genes was analyzed by cellulase activity assays and 12 % SDS-PAGE analysis. Recombinant clones showing the highest cellulase activity by filter paper assay (0.22 IU ml^{-1}), cellobiase assay (0.425IU ml^{-1}), carboxy methyl cellulose (CMC) assay (0.77IU ml^{-1}) and exoglucanase assay (0.40 IU ml^{-1}) were selected for further study. These recombinant yeasts were subjected to co-fermentation at 37^0C under anaerobic conditions in a bench-top fermenter using pre-treated straw as the sole carbon source. Samples were collected at 24 hour intervals for ten days and were tested for combined cellulase enzyme activity of the three recombinant *S. cerevisiae* by determining the amount of glucose produced by glucose oxidase assay. Highest glucose production was determined as 0.7 mg/ml on the third day (72 hours) of the fermentation. The production of ethanol was determined as 7.2g/100g pretreated straw using the dichromate method. Above clones will have the potential to produce ethanol by simultaneous saccharification and direct fermentation of cellulosic biomass.

Keywords: Ligno-cellulosic biomass, cellulose, hemicellulose, co-fermentation, filamentous fungi, second generation bio-ethanol

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