Cloning and expression of cellulase and xylanase genes of filamentous fungi in yeast for synergistic saccharification and direct fermentation of cellulosic biomass to ethanol

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ABSTRACT

The conversion of plant biomass into ethanol has been selected as a possible strategy for the effective reuse of natural resources providing a promising solution to the anticipated future fuel crisis in many countries. Second generation bioethanol production process involves pretreatment of plant biomass, enzymatic hydrolysis to reducing sugars and subsequent fermentation of reducing sugars into ethanol. Cellulase is an enzyme complex consisting of endoglucanase (EGL), cellobiohydrolase (CBH) and β-glucosidase (BGL) that act synergistically to convert cellulose, the most abundant renewable fraction of plant biomass, to glucose. Xylanases namely endoxylanase (EXN) and β-xylosidase (XYL) are responsible for depolymerization of xylan, the major constituent of hemicelluloses, to xylose. Among all microbial genera, filamentous fungi are known as good cellulases and xylanases producers. *Saccharomyces cerevisiae* can efficiently convert glucose into ethanol under anaerobic conditions but it cannot ferment xylose like pentoses. *Pichia stipitis* have the ability to utilize xylose to produce ethanol. Therefore, *S. cerevisiae* and *P. stipitis* were genetically modified with the objective of heterologous extracellular expression of cellulase and hemicellulase genes of filamentous fungi, capable of utilizing cellulose and hemicellulose (xylan) of plant biomass to produce ethanol. The three cellulase and the two hemicellulase genes were successfully characterized, cloned and expressed in *S. cerevisiae* and *P. stipitis* respectively. The genomic sequence and cDNA of β-glucosidase I (BGL1) and genomic sequence of endoxylanases I (EXNI) were isolated from *Trichoderma virens* and β- xylosidase (XYLI) were isolated from *Aspergillus niger*. Endoglucanase I (EGLI) and cellobiohydrolase I (CBHI) genes of *Trichoderma* were custom synthesized. The extracellular expression of all genes by recombinant yeast was successfully confirmed using SDS-PAGE and standard cellulase activity assay methods. BGLI activity expressed by the recombinant genomic clone was 3.4 times greater than that observed for the cDNA clone. Furthermore, the activity was similar to the activity of locally isolated *Trichoderma virens* confirming the successful approach of genetic engineering in the heterologous extracellular expression of BGLI using the (GAP) promoter driven expression system. The *S. cerevisiae* genomic DNA clone produced six times the amount of ethanol compared to the cDNA clone in fermenting cellobiose displaying the direct and/or indirect intron mediated enhancement (IME) effect in eukaryotic gene expression. Enzyme activities displayed by EGLI and CBHI harbouring recombinant *S. cerevisiae* were 0.77 IU mL^{-1} and 0.4 IU mL^{-1} respectively. Three potential N-linked glycosylation sites were identified in EXNI using NetOGlyc 4.0 Server and GlycoEP server in this study. The discrepancy (an increase) in the molecular weight observed for SDS-

PAGE analysis of EXNI compared to the theoretical molecular weight can be attributed to hyperglycosylation. Under optimum growth conditions, EXNI and XYLI enzyme activities expressed by the culture supernants of recombinant *P. stipitis* clones were 31.7 $IUmL^{-1}$ and 57.4 IU mL^{-1} respectively. Co-fermentation of pretreated straw by the three recombinant *S. cerevisiae* harbouring cellulases yielded 3.24% (w/w) ethanol whereas two recombinant *P. stipitis* harbouring xylanases yielded 2.3% of ethanol. The ethanol yield obtained in the co-fermentation of pretreated straw was obviously less than the yield obtained for pure crystalline cellulose (avicel) and pure xylan by recombinant yeast. The overall conversion of plant biomass (pretreated straw) to ethanol by both recombinant cellulase and hemicellulase harbouring yeast was 14.8% of the theoretical maximum of approximately 45% dry mass of cellulose and 35% dry mass of xylan fraction of pretreated straw.