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Development of recombinant *Pichia stipitis* habouring endoxylanase and endo-β-D-xylosidase genes of filamentous fungi for degradation of hemicelluloses

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Production of second generation bio-ethanol has become a key area of research and development in many countries since alternate fuel production has become crucial for economic development. Hemicellulose is the second most abundant naturally available polysaccharide in the biosphere. Xylan is a major component of hemicelluloses in plant biomass and it is a polymer of 1,4-linked β-D-xylose units. Xylan can be hydrolyzed to fermentable xylose using xylanases. Two major xylanases, namely endo-β-1,4-xylanase (EXN) and β-1,4-xylosidase (XYL) are needed for the complete hydrolysation of xylan. Endo-β-1,4-xylanases cleaves xylan to produce xylo-oligosaccharides and β-1,4-xylosidases hydrolyzes xylo-oligosaccharides to D-xylose. *Among all microbial genera* these enzymes are produced in considerable amounts by *two filamentous fungi species namely Trichoderma and Aspergillus. Among* yeast species, *Pichia stipitis*, *Pichia segobiensis,Candida tenius, Candida shehatae* have the ability to utilize xylose for their growth and are also able to ferment pentose sugars to ethanol.

The aim of this study was to isolate endo-β-1,4-xylanase (EXN) and β-1,4-xylosidase (XYL) genes respectively from *Trichoderma and Aspergillus* species, clone and express the enzymes in *Pichia stipitis* for the simultaneous saccharification and direct fermentation of hemicellulose into ethanol. Locally isolated *Trichoderma virens and Aspergillus niger were selected and tested for hemicellulase activity.* EXN and XYL genes were successfully PCR amplified from genomic DNA of *T. virens and A. niger,* respectively. Amplified genes were individually cloned into pGAPZα expression vector and then transformed into *P. stipitis.* Recombinants were confirmed by colony PCR and sequence analysis. Putative recombinants were designated as Y-pGAPZα/EXN and Y-pGAPZα/XYL. The expression of EXN and XYLby recombinant *P. stipitis* was confirmed using standard hemicellulase activity assay using xylan as the substrate and 12% SDS-PAGE analysis. The determined enzyme activities for Y-pGAPZα/EXN and Y-pGAPZα/XYL were 529.3 nkat ml^{-1} and 959.3 nkat ml^{-1} respectively. SDS-PAGE analysis revealed fragments of 24 kD for *EXN* and 84 kD for *XYL* confirming expression of the recombinant proteins. Further studies will be conducted to optimize the enzyme activities expressed by the two recombinant *P. stipitis* clones to degrade and ferment hemicelluloses into ethanol from pretreated straw by anaerobic fermentation.

Keywords: Second generation bio-ethanol, hemicelullose biomass, simultaneous saccharification

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