Co-expression and Secretion of Thermostable Laccase and Xylanase in *Pichia pastoris*

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The vegetal primary wall is composed of cellulose fibrils, hemicellulose, lignin and protein forming a matrix that cements the wall together. The enzymatic degradation of plant cell wall polymers has recently attracted attention in production of second generation biofuels. Xylanases (1,4-β-D-xylanhydrolase, EC 3.2.1.8) are endo-enzymes which hydrolyse the β-1,4-xylose glycosidic linkages of xylans, the principal polysaccharide of hemicelluloses. Laccases (benzenediol:oxygen oxidoreductase EC 1.10.3.2) play a role in the degradation of lignin, and can be included in the broad category of ligninases. In this work a broth containing thermostable laccase and xylanase was produced by co-expression of both enzymes in *Pichia pastoris* strain GS115. The vector pPIC9k was used for cloning the laccase gene and was inserted in the HIS4 gene and the pPICzalfa vector was used for cloning the xylanase gene and inserted in the GAP promoter locus, both by a single crossover event. The vectors are regulated by the AOX1 promoter which drives heterologous protein expression in *Pichia,* and the secreted heterologous proteins comprise the majority of the total protein in the medium. The transfected yeast was cultured in BMM medium containing 0.25 mM $CuSO_4$ and protein expression was induced by methanol. After 5 days induction high levels of protein were expressed. The activities of both enzymes were measured in pH6, the xylanase show maximum activity at temperature of 65^{o}C, and laccase exhibited a maximum activity at 75^{o}C. The xylanase shows thermotolerance with a half-life of 25 minutes at 65^{o}C and lacase was over 100 minutes. The catalytic properties were different when these enzymes were expressed in E. coli, and changes in mobility were observed in SDS-PAGE indicating that enzymes expressed in *Pichia pastoris* were glycosylated. These results demonstrated the viability of enzyme co-production to produce mixtures for biotechnology applications.

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