

Revolutionizing Bioethanol Production: Genetic Engineering of *S. cerevisiae* for the Single Step Conversion of Biomass to Bioethanol

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The goal of this research was to clone both the *lacc2* gene and the *cbhl* gene from *Pleurotus ostreatus* into the common yeast, *Saccharomyces cerevisiae*. The *Lacc2* coding sequence is 1.57 kbp long, encoding 506 amino acids, and the genomic sequence is interrupted by 21 introns, whereas the *cbhl* coding sequence is 1.5 kbp long, encoding 496 amino acids, and is interrupted by 3 introns. Both of the genes were first amplified from *P. ostreatus* cDNA (reverse transcribed from mRNA) using novel gene-specific primers, and were then ligated into the pGEMt-Easy vector and cloned into DH10B *E. coli* competent cells. *Lacc2*+pGEMt and *cbhl*+pGEMt were then digested by *Xho*I and *Xba*I restriction enzymes and ligated into the pYES2 yeast expression vector. *Lacc2*+pYES2 and *cbhl* +pYES2 were then subcloned into separate *S. cerevisiae* competent cells. Spectrophotometric enzyme activity assays were performed on the recombinant yeast cultures using ABTS and microcrystalline cellulase as respective substrates. The concentrations of expressed laccase and cellobiohydrolase were 89.5 U/mL and 151.3 U/mL. Biomass fermentations were performed using the recombinant yeast- the combined bioprocessing method oxidized the most amount of lignin, reducing lignin content by approximately 20% and hydrolyzed the most amount of hemicelluloses, reducing hemicellulose content by 26%. This yielded 1.24 mL per gram of lignocellulosic substrate, which outperforms the two most popular industrial methods, biological pretreatment and acid hydrolysis, by 2.5- fold by 26-fold, respectively.