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 cbhI 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 cbhI 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 cbhI+pGEMt were then digested by XhoI and XbaI restriction enzymes and ligated into the pYES2 yeast expression vector. Lacc2+pYES2 and cbhI +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.