Cloning and Sequencing of the Lacc2 Laccase Gene from the Ligninolytic Basidiomycete Pleurotus ostreatus

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Cellulosic bioethanol, an alternative energy source produced by saccharification and fermentation of plant polysaccharides such as cellulose and hemicellulose, is a promising renewable energy source that could replace fossil fuels. However, isolating these polysaccharides from lignin, an aromatic compound found in the plant cell wall, requires much energy input and time. Increasing research shows that white-rot fungi, such as the basidiomycete Pleurotus ostreatus can naturally oxidize lignin through extracellular oxidative enzymatic activity. Fungal laccases are lignin-modifying oxidoreductases that catalyze the oxidation of inorganic and organic aromatic compounds. This catabolic activity breaks down the carbon- carbon bonds in lignin. Therefore, the cloning of genes expressing lignin-modifying enzymes may have huge potential in streamlining the bioethanol process. The goal of this research was to attempt to clone and sequence the gene, Lacc2, which expresses the POXA3a laccase isoenzyme from the basidiomycete P. ostreatus. This gene was chosen because it expresses an isoform of laccase that is most active during the vegetative growth phase of P. ostreatus. The Lacc2 coding sequence is 1,569 bp long, encoding 506 amino acids, and the genomic sequence is interrupted by 21 introns. cDNA from P. ostreatus was PCR amplified with Lacc2 gene-specific primers and then sequenced. The PCR product was ligated into the pGEMt-Easy vector system and was then cloned into DH10B E. coli competent cells and plated on LB+Ampicillin media. PCR was then performed on the transformed Escherichia coli and the results indicated that a positive clone was present, confirming the experimental prediction that the Lacc2 gene could be cloned and amplified.

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