Studies of a Bacterial CotA Laccase Expressed in *Pichia pastoris*

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Introduction: The plant cell wall is a complex composite matrix constituted by cellulose, hemicellulose and lignin that is highly recalcitrant to depolymerization. The degradation of the plant cell wall is necessary for the use of vegetal biomass for production of second generation ethanol, and enzyme mixtures that include hydrolases and ligninases show promise for biomass decomposition. Laccases (EC 1.10.3.2) are components of the lignolytic complex responsible for lignin decomposition, and the present work the *Bacillus subtilis* laccase CotA was expressed in *Pichia pastoris*, purified and biochemically characterized. *Result*: the *cotA* gene was amplified from *Bacillus subtilis* genomic DNA and cloned in pPIC9k vector, under control of the AOX1 promoter. The transformed yeast was cultured in BMM medium containing 0.25 mM $CuSO_4$ and protein expression was induced by methanol. After 6 days induction high levels of protein expression (>15 mg/L) were obtained and the recombinant laccase was purified by Ni-affinity chromatograph. The oxidation of ABTS (2,2′-azinobis-3-ethylbenzthiazoline-6-sulfonate) by recombinant CotA exhibited a maximum activity at pH 5.2 at a temperature of 80^{o}C. The enzyme shows high thermotolerance with a half-life of 220 minutes at 80^{o}C. Analysis of the CotA by circular dichroism indicates a protein structure rich in β-sheet that is comparable to the same protein expressed in *E. coli*, showing that there were no significant changes in secondary structure between CotA expressed in bacteria and in yeast. However, changes in mobility in SDS-PAGE and in the catalytic properties indicate that CotA expressed in *Pichia pastoris* is glycosylated. *Conclusion*: This is the first report of a bacterial laccase expressed in yeast. The enzyme has a high expression, high activity against synthetic substrates and high thermotolerance, properties that are of interest for applications in biomass degradation.

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