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Cloning and expression of Laccase from *Bacillus subtilis*

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Background and Aims: Laccases are known as a large group of phenol-oxidizing enzymes widely distributed in nature among some of the plants, fungi and bacteria. Bacterial laccases are found highly active and stable at high temperatures and high-PH values. Laccase pharmaceutical and industrial applications include: biosensors for drug analysis, polymer synthesis, bioremediation, nanobiotechnology, food and cosmetic industries, catalyst for manufacturing of anticancer drugs, novel penicillins and cephalosporins. In this study laccase gene from Bacillus subtilis was cloned and expressed in Escherichia coli to achieve high-level production of the recombinant enzyme.
Methods: B. subtilis from PTCC was grown in LB medium at 37 °C and genomic DNA was extracted. The gene encoding laccase (CotA) was amplified using PCR with designed specific primers. The PCR amplicon was digested with BamHI and HindIII and ligated into expression vector pET28a. The resulting recombinant plasmid was used to transform the expression host Escherichia coli BL21 (DE3). The cells were induced with Isopropyl-β-D-1-thiogalactopyranoside (IPTG) and supplemented with CuSO4 to obtain the best condition of expression. Protein purification was carried out and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).
Results: The cloning process was verified by restriction digestion and sequencing. Nucleotide sequence analysis of recombinant plasmid confirmed an open reading frame of 1542 bp coding for a protein of 514 amino acid residues. SDS-PAGE analysis of cell lysate revealed a band of about 65 kDa and 6xHis-tagged protein was obtained after purification.
Conclusions: Bacterial laccases overcome disadvantages of fungal and plant laccases and bacterial expression system has the benefit of being relatively simple compared to fungal ones. High level production of recombinant laccase in E. coli BL21 (DE3) and simple purification of His-tagged protein suggested that this enzyme can be a potential candidate for biotechnological applications.

Keywords: Laccase; *Bacillus subtillis*; Cloning; Purification