Cloning and Expression of the para-Hydroxybenzoate Hydroxylase Gene from Pseudomonas aeruginosa and ...

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ABSTRACT

L-DOPA is a medicine to prevent Parkinson's disease getting worse, therefore para-hydroxybenzoate hydroxylase (HBHD)from Pseudomonas aeruginosa PAO1 was chosen to substitute for tyrosine hydroxylase to transform L-tyrosine become L-DOPA to achieve the treatment. So hbhd gene was used as template to design primer pair. Chromosome DNA of P. aeruginosa PAO1 was used as template to amplify hbhd gene by PCR. Cloning of hbhd gene into Escherichia coli and expression of enzymatic activities were performed. The length of hbhd performed open reading frame（ORF）was 1,185 bp. The translation HBHD product was 45 kDa of molecular weight. The P. aeruginosa hbhd gene was cloned into pQE30 expression vecter and transformated to E. coli Nova Blue. Finally, Ni-NTA column was used to purify the enzyme. With the para-hydroxybenzoate as subtrate, enzyme activity was maximal at 80 °C, the optimum pH 9.0 assayed by high performance liquid chromatography, Fe2+ ion promoted enzyme activity. When the concentration of para-hydroxybenzoate reached to 100 mM, enzyme activity was measured without any substrate inhibition. With the L-tyrosine as substrate, enzyme activity was maximal at 30 °C, the optimum pH 3.5 characterized by HPLC. Without metal ion, the HBHD is still active. When the concentrations of L-tyrosine and L-DOPA reached to 80 mM and 3.8 mM, the HBHD activity was reduced to 50 % with the substrate and product inhibition, respectively.

Keywords : L-DOPA ; gene cloning ; igh performance liquid chromatography

Table of Contents

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