Para-hydroxybenzoate hydroxylase 基因選殖、表現與酵素活性分析

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摘 要

左多巴(L-DOPA)為目前醫學上最普遍拿來當做治療帕金森氏症(Parkinson's disease)的藥物，故希望能從微生物內尋找tyrosine hydroxylase類似物para-hydroxybenzoate hydroxylase (HBHD) 以期將L-tyrosine轉換成L-DOPA而達到治療治療帕金森氏症。從Pseudomonas aeruginosa PAO1找出hbhd基因序列。將hbhd基因選殖入Escherichia coli 菌體內，可表現HBHD的活性。HBHD的open reading frame (ORF)全長為1185 bp，轉譯出HBHD蛋白分子量45 kDa。將P. aeruginosa PAO1之HBHD ORF選殖入pQE30，轉形至E. coli Nova Blue內，再用Ni-NTA管柱回收此酵素。經過高效液相層析法 (High performance liquid chromatography)分析酵素活性後，以para-hydroxybenzoate為受質，在80 ℃下反應，pH 9.0為最適反應，Fe2+有促進反應效果，其受質濃度達100 mM前，沒有受質抑制的效果，當產物達到1 mM就會產生產物抑制；以L-tyrosine為受質，在30 ℃下反應， pH 3.5為最適反應，對金屬離子沒有特別的促進或者抑制效果，其受質濃度達80 mM會有受質抑制現象發生，活性降為50 %，當產物達到3.8 mM，會有產物抑制現象，活性降為50 %。

關鍵詞 : 左多巴 ; 基因選殖 ; 高效液相層析法

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