Study and Evolution Cloning of the Gene Encoding the L-AsparaginaseII from E. coli in B. subtilis

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Abstract

***Background and purpose**: L-asparaginaseII (E.C. 3.5.1.1) has effective usage for treatment of acute lymphoblastic leukemia. This enzyme is isolated from bacterial sources and it is commercially available as an anti-cancer drug. This enzyme catalyzes the hydrolysis of L-asparagine to ammonium and aspartate. Unlike Normal cells, the cancer cells strongly require L-Asparagine leading to destruction of these cells. The propose of this project is cloning of L-asparaginaseII gene from E. coli in B. subtilis to produce the mass of this enzyme.*

***Materials and methods**: L-asparaginaseII gene is isolated from E. coli by PCR approach. The amplified fragment and expression shuttle vector pMR12 are digested by HindIII and BamHI enzymes. The ligation between DNA fragment and the cloning shuttle vector is done by standard method. In the next step recombinant vector is transferred to E. coli JM101 by cold calcium chloride treatment and finally introduced into B. subtilis by a chemical method.*

***Results**: in this experiment, ansB gene is isolated from E. coli by PCR and cloned into the expression shuttle vector pMR12. Existence of gene with 1047 bp lenght is confirmed by enzymatic analysis and PCR reaction. Then recombinant vector cloned in E. coli at first and then in B. subtilis. Finally the plasmid extracted and compared with the band of expersion shuttle vector containing ansB gene to confirm.*

***Conclusions:** In this study, we tried to clone the ansB gene in B. subtilis using expression shuttle vector pMR12. This is the first report of ansB gene cloning in B. subtilis.*

***Key words:** L-Asparaginase, E. coli, B. subtilis, Cloning, pMR12 .*

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