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Enhanced Production, Purification and Thermodynamic Characterization of Endotoxin Free Antileukemic Recombinant L-Asparaginase of *Escherichia Coli K-12*

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L-Asparaginase (E.C. 3.5.1.1) is a well accepted chemotherapeutic agent against the acute lymphoblastic leukemia and lymphosarcoma. The recombinant L-asparaginase enzyme was produced by the over-expression of ansB gene of *E. coli K-12* in *E. coli BL21*. Different carbon sources, nitrogen sources, minerals and additives having yield enhancing effect, were screened by Plackett Burman Design (PBD). Their optimum level was identified by the using the orthogonal array method of Taguchi design of experiment. The screening of media components by PBD proposed the glucose as main carbon source, tryptone and yeast extract as organic nitrogen source, NH_4Cl as inorganic nitrogen source, NaCl and K_2HPO_4 as mineral source have significant effect on the production of enzyme. Glycerol was identified as the most influential effect on the recombinant L-asparaginase production among the all additives. The addition of small amount (0.6% v/v) of it, significantly increased the enzyme yield. After the complete optimization of the selected process parameters 121.8% enhanced production of L-asparaginase was observed at shake flask level. There was further 14.8% enhancement in the enzyme production after the scale up the process in 5L bioreactor. The volumetric yield of 3.58 X 10^5 U/L of L-asparaginase with the specific activity of 6.97 X10^3 U/mg in fermentation broth was reported. The enzyme was purified by affinity chromatography followed by three-phase partitioning. The endotoxin level was estimated by LAL-assay. The purified recombinant enzyme was further used to study the thermodynamic parameters. The enzyme showed highest stability at 28^0C than at higher temperatures with a half-life of 46 hrs which is quite significant. Its deactivation energy was found to be 60.64 kJ/mol. The value of thermodynamic parameters including ΔH, ΔS and ΔG were found to be -49.23kJ/mol, 0.09kJ/mol.K and 73.12-74.78 kJ/mol respectively implying that there are no significant processes of aggregation and the enzymatic reaction was exothermic and spontaneous in nature. The antileukemic potential of the enzyme was accessed by using human leukaemia cell line HL-60 through viability study, cell and morphological studies.

Biography:

Dr. Santosh Kumar Jha is an academician cum researcher and presently serving as an Assistant Professor in the Birla Institute of Technology, Mesra, India. He has expertise in the process development and designing of bioprocess for the production of biotherapeutics. Presently he is working on the bioprocess development of various biotherapeutics, development of nanobiocomposite based nanobiotics and tissues scaffolds for various biomedical applications.