Parameter Optimization for Recombinant Asparaginase Production in *Escherichia coli*

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Abstract

L-Asparaginase enzymes (L-Asparagine amidohydrolase; EC 3.5.1.1) catalyze the hydrolysis of L-Asparagine (L-Asn) to L-Aspartate (L-Asp) and ammonia (NH$_3$), and to a lesser extent the hydrolysis of L-Glutamine (L-Gln) to L-Glutamate (L-Glu) (Wang *et al.*, 1999). Two types of bacterial L-asparaginases have been identified: type I and type II (Campbell *et al.* 1967). Type II L-asparaginases, in particular, present tumor inhibitory activity and have thus been extensively studied (Cedar and Schwartz 1968).

Tumor-inhibitory L-asparaginases have also been isolated from a number of bacterial sources. However, the enzymes from *Escherichia coli* (*E. coli*) and *Erwinia* sp. have been more frequently used in cancer therapy (Avramis and Panosyan 2005; Oettgen *et al.* 1970) due to their higher substrate affinity (Schwartz *et al.* 1966) and favorable factors affecting the clearance rate of these enzymes from the system (Broome 1965).

Media optimization for the production of enzymes is an important step for commercial applications and involves the study of a number of physicochemical parameters, such as medium composition, pH and temperature. Recently, the use of statistical approaches involving Plackett–Burman (P-B) designing and response surface methodology (RSM) have been widely used for media optimization and for the understanding of interactions among various parameters using a minimum number of experiments (Kalil *et al.*, 2000).

The aim of this work is the optimization of culture conditions in order to maximize the recombinant asparaginase production in *E. coli*.

After amplification of the asparaginase gene from *Erwinia carotovora*, the gene was cloned into the expression vector pET30a(+) and used to transform BL21(DE3) *E. coli* strain.
by electroporation. A master cell bank was done with 30% glycerol and kept at -20°C and used in all experiments. As control the plasmid lacking the asparaginase gene was used.

The culture medium were inoculated to an OD_{600nm} of 0.1 and incubed at the conditions defined by the Plackett & Burman statistical design. The optic density was monitored at each hour. After reaching an OD_{600nm} of 0.4 - 0.6, the cultures were induced with 0.1 mM IPTG. A control without IPTG was also done for each culture. For expression analysis 1 mL of the culture was collected

The 1 mL collected samples were used for protein expression analyses by eletroforesis in polycrilamide gel (SDS-PAGE). Samples were collected in time periods of: 0, 3, 6, 9 and 22 hours after induction. In order to separate the phases, the samples were centrifugated at 13000 rpm for 3 min. The precipitated and the liquid phase of the samples were frozen at -20°C.

To determine which nutrients and conditions had a significant effect on asparaginase production, P-B design was used (Plackett and Burman, 1946). In our work, eight variables and four dummy variables were screened in 12 trials, with four replicates in the central point. The variables studied were: (X_1) temperature from 27 °C to 37 °C; (X_2) yeast extract from 5 gL$^{-1}$ to 20 g L$^{-1}$; (X_3) tryptone from 5gL$^{-1}$ to 20gL$^{-1}$; (X_4) glucose from 0 to 10 g L$^{-1}$; (X_5) glycerol from 0 to 20 g L$^{-1}$; (X_6) medium M9 from 0 to 2 x; (X_7) tiamine from 0 to 2 x (2 µg/mL); (X_8) trace solution from 0 to 2 x. The initial pH of the media was 7.0 in all experiments.Variables with confidence levels >95% were considered to have significant influence on asparaginase production.

Microbiological growth will be measured throw optical density at 600 nm. The protein expression analyses will be controlled by eletroforesis in polycrilamide gel (SDS-PAGE), and quantified by densitometry. Analyses of pH will be done by a standard lab pHmeter.

With the results obtained from the Plackett & Burman statistical design will be possible to select the significant parameters for asparaginase production. After this step, the culture condition will be optimized through Central Composite Design (CCD). The optimization will be defined by the maximal quantity of biomass and protein, in the tested conditions.
References


