TITLE: L-ASPARAGINASE PRODUCTION FROM *E. coli* BL21 (DE3) COMPARING LACTOSE AND IPTG AS INDUCERS.

AUTHORS: BARROS, T.S.[1]; ALMEIDA,R.P.C.[1]; ANDRADE, K.C.R.[1]; CARDOSO, S. L.[1]; SOUZA, P.M.[1]; PESSOA, A.[2]; PARACHIN, N. S.[3]; MAGALHÃES, P. O.[1]

INSTITUTION:

[1] Laboratory of Natural Products, Health Science School, Department of Pharmacy, University of Brasília
[2] Department of Pharmaceutical and Biochemical Technology, University of São Paulo
[3] Grupo Engenharia de Biocatalisadores, Instituto de Ciências Biológicas, Universidade de Brasília

ABSTRACT

Escherichia coli is a bacteria widely used for the production of recombinant proteins, because its metabolism is known from an efficient expression platform. In this work, a strain of *E. coli* BL21 (DE3) was used to produce the enzyme L-asparaginase (ASNase). The ASNase enzyme has been widely used in the treatment of acute lymphoid leukemia. Cancer cells are unable to synthesize asparagine, thus the L-asparaginase hydrolyze the amino acid L-asparagine in L-aspartic acid and ammonia and depletes the supply of asparagine in the blood plasma, leading to a selective apoptosis of cancer cells. The expression vector pET15b used in this work can be induced by lactose or by IPTG. The objective of this work is to compare the induction by lactose and IPTG in the L-asparaginase production. For this, *E. coli* was grown in defined medium with the following composition: glucose, KH_2PO_4, $MgSO_4$, H_3BO_3, Na_2MoO_4, $CoCl_2$, $(NH_4)_2HPO_4$, citric acid, iron citrate III, $MnCl_2$, $Zn(CH_3COO)_2$, $CuCl_2$, $EDTA.Na_2$ and thiamineHCl. The cultures were incubated on a shaker at 37 °C and 180 rpm. An ASNase activity was measured by the L-aspartyl-β-hydroxamic acid in suspension of 50 mg/mL of moist cells. Four concentrations of lactose (6, 10, 14 and 18 g/L) and three concentrations of IPTG (1, 0.45 and 0.1 mM) were tested. After defined the best concentration of the inductors, also it was tested the best time to start (3, 5, 7, and 9h) and stop (10-24h) the induction. The best concentration of lactose was 10 g/L, with the activity of 76 U/g_{cell}. For IPTG the best concentration was 0.45 mM, with an activity of 112 U/g_{cell}. The point that presented the best induction time for the two inductors was 5 hours, where lactose presented the activity of 78 U/g_{cell} and IPTG of 112 U/g_{cell}. In the time evaluated after the lactose induction, the best activities were found between the points of 18h and 20h, with no statistical difference, with the activity of 99 U/g_{cell}. IPTG concentrations made up a plateau between the points from 16h to 24h with no statistical difference, with the mean of activities being 109 U/g_{cell}. For induction of 1 L of medium the amount spent with IPTG is $6.60 and lactose is $3.56. Aiming at industrial scale cultivation, the use of lactose as an inducer is interesting. The values of asparaginase activity in the best times are close and the final cost of lactose is lower than IPTG, thus making viable the use of lactose.

Keywords: Leukemia, L-asparaginase, induction, Lactose, IPTG.

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