Title: Study of the induction time of L-asparaginase II from *Saccharomyces cerevisiae* expressed in *Pichia pastoris* (Mut^{s})

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Abstract:

Asparaginase (EC. 3.5.1.1) is a therapeutic enzyme used for the treatment of acute lymphoblastic leukaemia and non-Hodgkin lymphomas. Bacterial L-asparaginase is produced in large scale, but shows several immunological reactions. Because of this problem, novel systems from eukaryote microorganisms have been investigated to produce this enzyme. *Saccharomyces cerevisiae* is able to express two types of L-asparaginase: intracellular and fixed in the cell wall. Some characteristics as stability and optimum pH, presented by asparaginase II, can be an important alternative as antitumoral agent. The methylotrophic yeast *Pichia pastoris* can be cultivated at very high biomass concentrations and it is an important option to produce large quantities of recombinant proteins controlled by the methanol-inducible AOX-promoter. Glycerol can be used as a substrate for biomass production during the first phase of cell growing, followed by second phase in which the methanol is used for inducing expression of the desired protein. In this study, the culture was carried out in 250 mL Erlenmeyer flask containing 50 mL of BMGY (pH 6.0) medium with 10 $g.L^{-1}$ of glycerol at 30°C, 250 rpm and 1 $g.L^{-1}$ of inoculum. After glycerol depletion, the cells were harvest by centrifugation at 3000*xg* and 10°C for 10 minutes. The pellet was resuspended in 50 mL BMMY (pH 6.0) medium. The induction with methanol 0.5% (v/v) was performed every 24 hours during 72 hours. The asparaginase activity was measured in whole cell suspensions (periplasmic activity) by the method based on asparagine hydroxylaminolysis. The glycerol was depleted after 12 hours of cultivation and the kinetics parameters of growth (μ_{max} = 0.35 h^{-1} and *tg* = 2.0 h) were calculated. Moreover, conversion factor ($Y_{x/s}$ = 0.88 $g.g^{-1}$) is very close to maximum value (0.9 $g.g^{-1}$) descripted to fermentation process using glycerol as the carbon source. The highest production of L-asparaginase (7.8 $U.g^{-1}_{dry\ biomass}$ activity) and dry biomass (12 $g.L^{-1}$) were obtained after 48 hours of induction. Finally, it can be enhanced that the recombinant L-asparaginase expressed by *P. pastoris* and the native one expressed by *S.* cerevisiae are inserted into the cell wall structure.