TITLE: EXPRESSION OF HETEROLOGOUS L-ASPARAGINASE FROM *Fusarium proliferatum IN A Pichia pastoris X33 strain.*

AUTHORS: CARDOSO, S.L[1].; SANTOS, L.R.C.[1].; SOUZA, P.M[1]; MARTINS, D.H.N[1]; ALMEIDA, R.P.C[1]; BARROS, T.S.[1]; PESSOA, A[2].; 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

L-asparaginase catalyzes the hydrolysis of L-asparagine to L-aspartate and ammonia. It has been used as an effective drug in treatment of acute lymphoblastic leukemia. Neoplasic cells cannot synthesize L-asparagine unlike normal cells due to the low expression or absence of the L-asparagine synthetase gene, therefore they obtain the required asparagine from circulating pools. *Pichia pastoris* has been successfully used for the expression of some heterologous proteins of biotechnological interests. This work evaluated the system of proteins heterologous expression using the yeast *P. pastoris* for L-asparaginase production, in order to obtain it in larger amounts for their subsequent biochemical characterization and application in biotechnological processes. The L-asparaginase cDNA isolated from *Fusarium proliferatum* was inserted into *P. pastoris* genome using vector pPICZα, based in the AOX promoter, and grown under constant agitation for L-asparaginase production. Asparaginase assay was performed for the formation of β-aspartyl hydroxamate. The heterologous L-asparaginase was addressed to the *P. pastoris* cell periplasmic space. The results showed an enzymatic activity of 2.82 IU/g in a 50 mg/mL of cells suspension. The cloned strain demonstrated a functional enzyme and a promisor microorganism to produce heterologous L-asparaginase in a high level. More studies need to be done to optimization the production.

Keywords: L-asparaginase, *Pichia pastoris, Fusarium proliferatum.*

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