INDUCTION OF THE EXPRESSION OF GLYCEROL 3-PHOSPHATE DEHYDROGENASE IN *PICHIA PASTORIS*

Silva, V.C.¹, Apponi, L. H.², Peres M.F.S.¹, Valentini, S. R.², and Gáttas, E. A. L.¹

¹Departamento de Alimentos e Nutrição, ²Departamento de Ciências Biológicas, Faculdade de Ciências Farmacêuticas UNESP - São Paulo State University, Rodovia Araraquara-Jaú, Km 1, 14801-902, São Paulo, Brazil. e-mail: viviane@fcfar.unesp.br

The enzyme glycerol 3-phosphate dehydrogenase (NAD: oxido-redutase EC. 1.1.1.8) catalyzes the reduction of the glycolytic intermediate dihydroxyacetone phosphate to glycerol-phosphate in a reversible way. In the present study, the expression system EasySelect[®] (Invitrogen) in *P. Pastoris* was used with the purpose of inducing the extracellular expression of glycerol 3-phosphate dehydrogenase. The functionality of this enzyme obtained in culture medium was assayed as described by Gattás et al., r^oPI 025552-0, 2002. The induction of protein was determined in growth medium containing methanol BMMH (by Invitrogen corporation web page). The efficiency of the expression system was evaluated in cultures induced for 72 hours. An yeast clone showing the highest enzymatic activity in 24 hours was selected. Other growth media were tested and the medium containing sorbitol (100mM potassium phosphate, pH 6.0, 1.34% YNB, 4 x 10^{-5} % biotin, 1% sorbitol) was equivalent to the medium containing methanol.

Supported by FAPESP, PADC/UNESP