EXPRESSION OF PROTEIN TYROSINE PHOSPHATASE FROM SOYBEAN (*GLYCINE MAX*) IN YEAST

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Protein tyrosine phosphatases (PTP) are ubiquitious enzymes that play an important role in many physiological processes, including regulation of transduction cascades via MAPKs and other receptor-mediated processes involving phosphorylation-dephosphorylation cycles. The cDNA encoding one of the soybean PTP isoforms was amplified by PCR reaction after reverse transcription from total RNA extracted from cotyledons. The fragment obtained was cloned into the expression vector pYEX-4T1, which allows the expression of PTP as a fusion protein with the GST (Glutathione S-Transferase) tag in *Saccharomyces cerevisiae*. Protein synthesis was monitored by SDS-PAGE after induction by addition of Cu^{2+} at the log phase growth of *S. cerevisiae*. We observed that expression levels of PTP after Cu^{2+} was low even when cells were incubated for as long as 3h. This resulted in low amounts of soluble PTP after purification by GST-affinity chromatography. Thus, to improve protein yield and to facilitate protein isolation and purification, the PTP gene was subcloned into pPic9K vector, for secreted expression in *Pichia pastoris.* We are currently characterizing *P. pastoris* cells carrying multiple copies of pPic9k-PTP to begin expression analysis.

Financial Support: CAPES, CNPq, FAEPEX/UNICAMP and FAPESP