Studies of polygalacturonases from *Leucoagaricus gongylophorus*

Golfeto, C.C.; Adalberto, P.R.; Iemma, M.R.C.; Cass, Q.B.; Souza, D.H.F.

1Departamento de Química, Universidade Federal de São Carlos, São Paulo, Brazil

**INTRODUCTION.** The literature shows that pectinases from *Leucoagaricus gongylophorus* are good targets in the ants control. In this work we studied native and recombinant polygalacturonases (PGase) from *L. gongylophorus.*

**MATERIAL AND METHODS.** To obtain the recombinant PGase, the total RNA of fungal mycelium, with has grown in presence of poligalacturonic acid, was extracted and converted in cDNA. The ORF encoding PGase was cloned into pGEM vector, sequenced and subcloned into pETSUMO expression vector. Rosetta(DE3)pLysS and BL21(DE3)pT-groE E.coli strains were used to express the recombinant PGase. Were evaluated different factors that influence expression and solubility of recombinant protein, such as several IPTG concentrations, different temperatures and cell lysis conditions. To isolate the native PGase, extract containing secreted enzymes was precipitated with ammonium sulfate, applied to a gel filtration column and the fractions containing activity were analyzed in SDS-PAGE electrophoresis.

Kinetic parameters ($V_{max}$ and $K_{M}$), optimum pH and temperature of the PGase were determined. **RESULTS AND DISCUSSION.** Both E.coli strains tested were able to express the recombinant PGase although mostly in insoluble form. IPTG concentrations were varied from 0,02 to 1mM and two temperatures were used (37°C and 20°C) but the most protein remained as precipitate. Refolding of the fusion protein was tried by dialysis with gradient of decreasing concentration of urea however little soluble protein was recovered. Analysis in silico showed glycosylation sites in protein sequence and this must be the reason of the protein is insoluble.

Native PGase precipitated with 30-70% NH$_4$(SO$_4$)$_2$ and purified by Superdex-75 column was optimally active at 60°C and pH 5.0. Apparent $K_{M}$ and $V_{max}$ values were 0,5□mg.mL$^{-1}$ and 5,2□µmol.min$^{-1}$.mL$^{-1}$, respectively. **CONCLUSIONS.** Recombinant PGase from *L. gongylophorus* was expressed in E.coli but despite several attempts the protein remained insoluble, maybe due to the lack of glycosylation. Native PGase was isolated and enzymatically characterized.

Key words: *Leucoagaricus gongylophorus*, poligalacturonases, symbiosis

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