FUNCTIONAL EXPRESSION OF *Carica papaya* LIP1 LIPASE IN *Pichia pastoris*

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Introduction. Lipases are lipolytic enzymes that catalyze the hydrolysis of the ester bounds from triglycerides In addition, under certain reaction conditions; they also catalyze diverse types of synthesis reactions with potential applications in different fields like food, pharmaceutical and detergent industry (1). Lipolytic activity in *Carica papaya latex* has been reported since 1925, but up to now, only one lipase was identified in latex (2). Efforts to isolate this protein from the latex polymeric matrix have been unsuccessful. In order to overcome this problem heterologous expression of this protein presents an interesting alternative with the purpose of characterized and study the catalytic performance of this protein towards different synthesis reactions.
The aim of this work was to functionally express *Carica papaya* lipase Lip1 gene (GenBank ID: CBW44734.1) (2). as a functional protein using *Pichia pastoris* as an expression system, and to evaluate some of the properties of the enzyme.

Methods. Cloning was performed according to (3) using the constitutive promoter pGAPZαB. Growth and enzyme production was carried out at 30°C in YPG medium. Activity determination was carried out by titration in the hydrolysis of triglycerides.

Results. Surprisingly, *C. papaya* Lip1 (CpLip1) presented homology with microbial lipases. Important characteristics of the protein were deduced by this way, the residues from the catalytic triad (Ser289, Asp352 and His446) and the oxyanion hole residues (Ser207, Gly290) are highly conserved. A 29 amino acid signal peptide was predicted using the Expasy SignalP V4.0 program. Furthermore, the NetNGlyc 1.0 Server predicted five possible N-glycosylation sites at residues 35 (Asn, Lys, Ser, Phe), 89 (Asn, Arg, Tyr, Pro), 153 (Asn, Pro, Ser, Phe), 181 (Asn, Lys, Ser, Asn) and 453 (Asn, Ser, Thr, Leu) respectively.

After cloning, the maximal lipolytic activity of CpLip1 (0.45 U/mL in hydrolysis of pNPB) was reached between 72h and 96h of growth. Concerning the activity towards triglycerides, CpLip1 reaches the highest activity of 3.7±0.26 U/mL when olive oil was employed as a substrate (figure 1).
CpLip1 exhibited activity in a rather narrow pH range of 3 to 11 with a maximal activity achieved a between pH 8 and pH 9 and an optimal at pH 8.5.

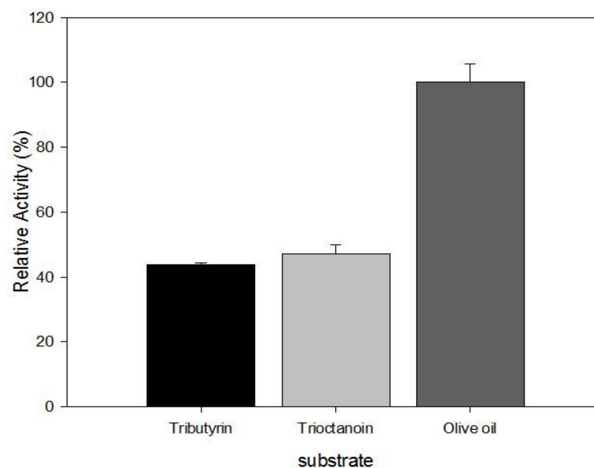

Fig 1. Substrate specificity of recombinant CpLip1 extract. Activity determinate by hydrolysis of olive oil (2.5 mL 20% olive oil emulsion, 1mL 50 mM Tris-HCl pH 8.5, buffer 35°C)..

Lipase activity of CpLip1 was also tested in a temperature range from 30°C to 70°C using olive oil as a substrate, in which the optimum temperature was obtained at 35°C.

Conclusions. This is the first report of functional expression of *Carica papaya* lipase (CpLip1). Indeed, previous efforts for expressing this protein in *E. coli* were unsuccesful (2). In this work, CpLip1 was successfully cloned and functionally expressed for the first time using *P. Pastoris* as an expression system using pGAPZαB as constitutive promoter. The recombinant lipase CP lip1 presents an optimal pH of 8.5 which is interesting for its application for instance in detergent industry.
CpLip1 capacity to hydrolyze long chain triglycerides such as olive oil make it attractive to test it is catalytic properties in lipid transformations.

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