RECOMBINANT PROTEASE FROM *Jacaratia mexicana* IN *Pichia pastoris*

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Key words: Jacaratia mexicana, Expression, Pichia pastoris

Introduction. Cysteine proteases (CPs) are important because they participate in key physiological processes and in mammalian cells are involved in pathological process (1). Plant CPs are used in the brewing, food and pharmaceutical industries (2) because they are considered GRAS enzymes and exhibit enzyme activity over a wide range of temperatures and pHs (3). *Jacaratia mexicana* is a Mexican endemic plant that contains cysteine proteases (members of the papain CI family). These enzymes have high enzymatic activity than papain (4). Recent studies have shown the expression of pro-enzyme encoded by the JmCP4 gene (isolated from *J. mexicana*) using *Pichia pastoris GS115* (Invitrogen).
The aim of this work was to obtain a functional recombinant protease from *J. mexicana* in *P. pastoris.*

Methods. Expression of JmCP4 gen in *P. pastoris* GS115 was performed according to the instructions of Pichia Expression Kit manual (5) with slights modifications (6). A single colony of yeast transformed with the vector pPIC9-JmCP4 (previously constructed by our working group and cloned in *E. coli* Top 10 F') was incubated into 50 mL BMGY medium in 250 mL flask with baffles and cultured at 30 ºC on a rotatory incubator at 250 rpm until reached OD_{600} 2.0. Cells were harvested by centrifugation at 6000 *g* and resuspended in 50 mL BMMY medium. The expression of JmCP4 was induced at 30 ºC for 144 h by adding methanol (0.5% of the workload) at 24 h intervals. Aliquots of the induced culture were withdrawn every 24 h. The expression of recombinant enzyme in culture medium was monitored by SDS-PAGE and protein by Bradford method (7). Enzyme activity was assayed using a synthetic substrate Nα-Benzoyl-L-arginine 4-nitroanilide hydrochloride (BAPNA) at pH 7.0 and zymograms with gelatin 0.5% (w/v) at pH 7.6.

Results. The insertion of JmCP4 gene of *J. mexicana* in the genome of *P. pastoris* was confirmed by sequencing and demonstrated the correct transformation of yeast. SDS-PAGE assay showed the overexpression of a pro-enzyme of apparent molecular mass of 32 kDa that was secreted into de medium. This pro-enzyme was detected by polyclonal Ab (anti-mexicain). The recombinant protease recovered from culture medium displayed enzymatic activity on gelatin and synthetic substrate BAPNA.

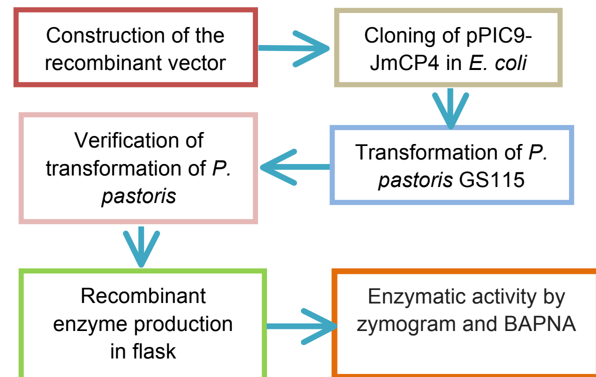

Figure 1. Experimental strategy to obtain a recombinant protease in *P. pastoris* GS115 pPIC9-JmCP4.

Conclusions. Overexpression of a functional cysteine protease of *J. mexicana* in flask was achieved in *P. pastoris* GS115 JmCP4.

Acknowledgements. Projects SIP-IPN 20121097 and 20131819. SIBE-COFAA.

References.

1. Mello V, Gomes M, Lemos F, Delfino J, Andrade S, Lopes M, Salas C. (2008). *Phytomed*. (15): 237-244.
2. Peng S, Zhu J, Li H, Tian W. (2008). *Bioscience*. Vol. (33): 681-90.
3. Dubey V, Pande S, Singh B, Jagannadham M. (2007). *Biotechnol*. (6): 1077-1086.
4. Oliver M. (1999). *Tesis de doctorado*. ENCB-IPN.
5. Herrera-Ramírez A. (2012). *Tesis de maestría*. UPIBI-IPN.
6. Pichia Expression Kit (Invitrogen, USA) 2010.
7. Bradford M. (1976). *Analyt. Biochem.* (72): 248-254.