PEPTIDASE-LIPASE BIFUNCTIONAL ENZYME EXPRESSED IN *Pichia pastoris*

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The protein engineering is a shortcut to natural evolution, one of the possible paths is the creation of hybrid enzymes or also called chimeras. These hybrids are the combination of two or more protein sequences, in order to improve or generate new functionalities. The peptidases and lipases are hydrolases capable of hydrolyze various substrates and have combined applications in different fields, as in the food, detergent, leather industries and bioremediation. End-to-end technique was employed to fuse the hydrolases sequences, the main advantage of its use is the independence of protein conformations and structures when compared to other techniques, making the process simpler. The chimera sequence was composed of hydrolases from *Fusarium oxysporum.* The peptidase was based on the work of Di Pietro et al (1998), which presented a subtilase, and the lipase sequence was based on the similarity with *Thermomyces lanuginosus* lipase. The position of each enzyme in the chimera was decided based on the in silico analysis of similar structures, where a His-tag was attached to the N-terminal region of the peptidase followed by a linker composed of five amino acids (GGAGG), and then the lipase sequence. The chimera gene was synthetized by GenScript® in pPiczαA expression vector for *Pichia pastoris* yeast.

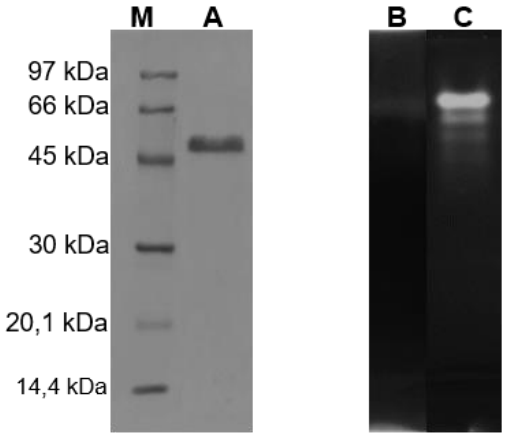

Figure 1 – Protein profile of in situ activity of bifunctional enzyme purified fraction produced by P. pastoris. (A) SDS/PAGE 12%; (B) In situ proteolytic activity with 0,1% gelatine as subtrate; (C) In situ lipolytic activity with 100 µM 4-methylumbelliferil butyrate as subtrate; (M) Marker LMW (Ge Life Sciences).

The chimera purification was performed using a Tricorn 5/50 column filled with Ni Sepharose™ excel resin coupled to the Akta Purifier UPC 900 system. The activity of peptidase and lipase were verified by zymogram protocols based on Marokházi (2004) and Prim et al (2003), using gelatin and 4-methylumbelliferil butyrate as substrates for each hydrolase, and the profile of the purified chimera was performed according to Laemmli (1970), as observed in Figure 1. The peptidase in the chimera was biochemically characterized according to Sarath et al. (1996) protocol with azo-casein 1% as substrate. Its optimum pH and temperature were determined as 6.0 and 50 °C. The stability in pH showed, after 1 hour of exposure at 25 °C, the maintenance of 70% of activity between pH 4.0-9.0. The stability of temperature was determined by exposing the enzyme during 60 minutes at a range from 30 to 60 °C, the activity was maintained above 50% from 30-45 °C. The effect of inhibitors testifies that the peptidase belongs to the serine peptidase class and it is dependent of ions for the catalytic activity, since it was inhibited by PMSF (phenylmethylsulfonyl fluoride) and EDTA (ethylenediaminetetraacetic acid). The ions effect assay showed the positive influence of different types of ions, such as monovalent ions – Li^+, Na^+ and K^+ – and divalent – Mn^{2+}, Mg^{2+} and Ba^{2+} -, but Ca^{2+} presented the major increase in the proteolytic activity of 75%. This bifunctional enzyme showed promising activity to different applications, proving the possibility of its use.

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Di Pietro, A. et al. Molecular Plant Pathology, v. 4, n. 5, p. 315-325, 2003.
Laemmli, U. K. Nature, v. 227, 1970.
Marokházi, J. et al. Applied and environmental microbiology, v. 70, n. 12, p. 7311-7320, 2004.
Nixon, A. E. et al. Trends in Biotechnology, v. 16, n. 6, p. 258-264, 1998.
Prim, N. et al. Journal of Molecular Catalysis B: Enzymatic, v. 22, n. 5–6, p. 339–346, 2003.
Sarath G.et al. Proteolytic enzymes: a practical approach, 2001