BIOCHEMICAL CHARACTERIZATION OF TWO THERMO-ALKALINE CUTINASES, ANCUT2 AND A NOVEL CUTINASE (ANCUT1) FROM *ASPERGILLUS NIDULANS*

Eva Bermúdez-García, Fernanda Vega-Pérez, Carolina Peña-Montes and Amelia Farrés; Department of Foods and Biotechnology, Faculty of Chemistry, National Autonomous University of Mexico (UNAM), Mexico City; evabermudez5@gmailcom

Key words: Aspergillus nidulans, cutinase, purification, thermostable, alkaline

Introduction. Cutinases are enzymes that carry out the hydrolysis of carboxylic acid esters in the cutin polymer (1), a structural lipid component of plant cuticle (2). This type of enzymes also performs the hydrolysis of small esters and polyesters. They show great potential in many biocatalytic processes, including biodiesel production (3, 4). Four genes that encode putative cutinases have been identified in the genome of *Aspergillus nidulans* (5). In this work, we describe the purification and detailed biochemical properties of two of the four cutinases, the previously reported ANCUT2 (6) and the novel ANCUT1.

Methods. *A. nidulans* was grown in optimized medium for the production of each cutinase, using as inducer: cutin for ANCUT1 and olive oil for ANCUT2. The supernatant was obtained by vacuum filtration and it was concentrated using ammonium sulfate precipitation for ANCUT1 or ultrafiltration for ANCUT2. A continuous elution denaturing electrophoresis (CEDE) was carried out to purify the enzymes. Their purity and activity was checked by SDS-PAGE, zymograms and quantitative activity against *p*-NPA as previously described (6). Enzymes were characterized in function to optimum pH and temperature under assayed conditions, stability against pH and temperature, substrate preference, effect of metal ions and surfactants and stability against solvents.

Results. ANCUT1 and ANCUT2 were obtained at a molecular weight of 22 and 29 kDa respectively and they retained their esterase activity after purification (Fig 1).

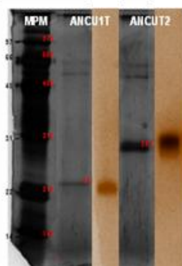

Fig.1 Purification of both cutinases and "in situ" activity by silver stainig of SDS-PAGE and zymograms.

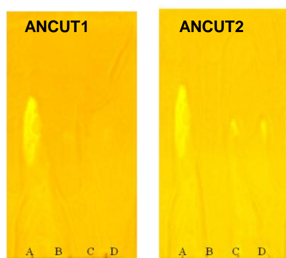

Fig.2 Products obtained after cutinolytic assay. Lanes: A: palmitic acid, B: blank reaction. C, D: enzyme reaction.

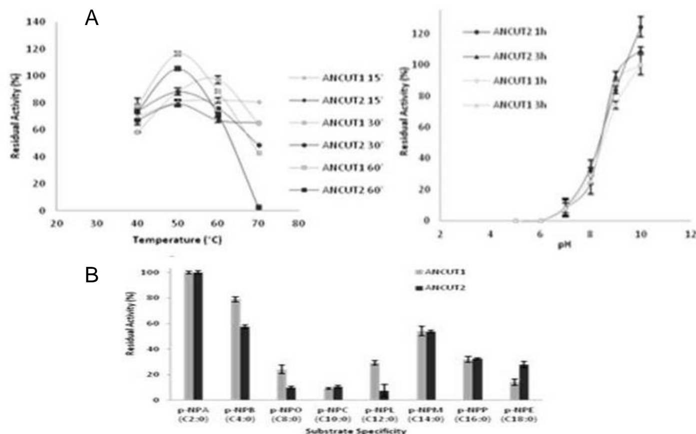

Fig. 3 Biochemical characterization of ANCUT1 and ANCUT2. A, Stability against temperature and pH. B, Substrate preference.

.

Both cutinases were thermo-alkaline (Fig 3A). An important difference between they was their substrate preference (Fig 3B) and the fact that only ANCUT2 was able to hydrolyze cutin, releasing palmitic acid (Fig 2). They presented an acceptable stability against non polar solvents; however ANCUT1 is more stable than ANCUT2. Metal ions showed no remarkable effect in the activity of both enzymes while surfactants reduced it. PMSF did not inhibit it completely as was expected because of the nature of the catalytic site.

Conclusions. The biochemical properties of the thermo-alkaline enzymes ANCUT1 and ANCUT2 make them suitable for potential biotechnological and industrial applications and show they have different physiological roles.

Acknowledgements. E. Bermúdez received a scholarship from CONACyT. This work was supported by CONACyT (153500) and DGAPA (IN231311).

References.

1.Carvalho C (1998), *Electron. J. Biotechnol* 1(3): 160-173.
2.Kolattukudy PE (1980), Cutin, suberin and waxes. In: *The biochemistry of plants, 4: Lipids: Structure and Function,* Stumpf PK, Academic Press, USA 571-645.
3.Jeong GT, Park DT (2008), *Appl Biochem Biotechnol* 148(3):131–139.
4.Bornscheuer, UT(2002), *FEMS Microbiol Rev* 26(1):73-81
5.Galagan JE, Calvo SE, Cuomo C, *et al.*, (2005), *Nature* 438:1105-1115.
6.Castro-Ochoa, et al, (2012), *Appl Biochem Biotechnol,* 166 (5), 1275-1290.