CLONING AND EXPRESSION OF A HALOPHILIC CARBOXYLESTERASE FROM THE ARCHAEON *Halobacterium* sp NCR1.

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Introduction. Carboxylesterases (EC 3.1.1.1) from archaeal sources are currently attracting enormous attention because of their biotechnological potential in medicine, synthetic chemistry and food processing (1). These applications are generally focused on the hydrolysis of esters. However, adding alternative nucleophiles, such as alcohols, amines and thiols, leads to transesterification, aminolysis and thiotransesterification, respectively. Halophilic enzymes are adapted to work in very high salt concentrations (with water molecules linked by ions); hence they are considered as potential catalyst for synthesis reactions using non-aqueous solvents (2). Camacho *et al* investigated the existence of esterases in the halophilic archaeon *Halobacterium* sp NCR1, finding that it produces these enzymes in small quantities (3), hence molecular biology approaches are needed to obtain this protein in larger quantities.
The aim of the present work was the clonning and expression of a halophilic putative Carboxylesterase (CEH) from *Halobacterium* sp NCR1 in *E. coli*.

Methods. *Escherichia coli* BL21 (DE3) Star was used for plasmid insertion and protein expression. The *Halobacterium* sp NCR1 genome was sequenced by Ng et al (3) and a putative CEH gene was searched using the KEEG (Kyoto Encyclopedia of genes and genomes, http://www.genome.jp.keeg/). The CEH gene was synthesized by the phosphoramidite method and inserted into the plasmid pET24a. This plasmid was used to transform *E. coli* and the transformed bacterium was selected on the base of kanamicine resistence. The protein expression was carried out using 0.1 mM isopropyl-β-D-thiogalactoside (IPTG) after 4 h of incubation at 30 ºC.

Results. The CEH gene (VNG1474G) encode for a protein of 274 amino acids with a molecular weight of 29.91 kDa. The expressed halophilic protein contained a large number of aspartic acid residues, migrating anomalously in a SDS PAGE, with an apparent molecular mass of 38 kDa (Fig. 1). This aberrant migration has been explained in others works due to the excess of negative charges on the surface of the protein (5). However, the recombinant protein was expressed by *E. coli* into inclusion bodies and consequently, the halophilic enzyme was inactive. Actually, we are working to disaggregate them using several strategies. The disaggregated protein will be purified by affinity chromatography and refolded with high salt concentration, in order to recover, the active enzyme.

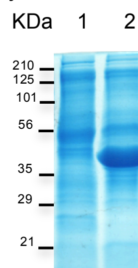

Fig. 1. SDS-PAGE for intracellular contents of the recombinant *E. coli* . Lane 1, non induced strain; lane 2, induced strain with IPTG for esterase synthesis.

Conclusions. We successfully cloned the CEH gene from the archaeon *Halobacterium* sp NCR1 into *E. coli* BL21 (DE3) Star. However, this recombinant enzyme was obtained as insoluble inclusion bodies, which are been treated to recover the activity.

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