IDENTIFICATION AND CHARACTERIZATION OF A NOVEL PEPTIDOGLYCAN HYDROLASE OF *Enterococcus faecalis* ISOLATED FROM COTIJA CHEESE®

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Introduction. Artisanal Cotija cheese® is produced from whole raw milk. No thermal step, neither starter cultures are used in the manufacturing process. After a three-month ripening stage, there is a decrease in coliform bacterial counts that could be caused by the production of antibacterial compounds by ripening bacteria, such as *Enterococcus* spp. (1). Enterococci may produce compounds such as bacteriocins or peptidoglycan hydrolases (PGH) (2). PGH are enzymes that hydrolyze the peptidoglycan, the bacterial cell wall main component (3). In our group, an *Enterococcus faecalis* strain was isolated from Cotija cheese®. It produces extracellular proteins with antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The aim of this work was to identify, clone, express and characterize the extracellular proteins responsible for such activity.

Methods. The extracellular proteins produced by *E. faecalis* cultured in MRS broth were precipitated using $(NH_4)SO_4$ (40% w/v) followed by CCl_3COOH (10% w/v). Its molecular mass was determined by SDS-PAGE and zymograms (4) against *Staphylococcus aureus*, *Escherichia coli* and *Micrococcus lysodeikticus*. Bands with bacteriolytic activity were selected and identified by LC-MS/MS. Their coding genes were amplified by PCR, cloned and expressed in *E. coli* BL21(DE3), using the pET-22b(+) as cloning vector and IPTG as inducer. The recombinant enzymatic activity was characterized by means of optimum temperature, thermal stability, effect of pH, ionic strength and presence of cations, using the Sigma β-*N*-Acetylglucosaminidase assay.

Results. Zymogram showed a 95-kDa band that was active against the three indicator microorganisms which corresponded to two different peptidoglycan hydrolases by LC-MS/MS. One of these was a putative *N*-acetylglucosaminidase of 54 kDa. It was cloned and expressed in *E. coli*, with a molecular mass of 75 kDa (Fig. 1). It showed *N*-acetylglucosaminidase activity, with an optimum temperature of 50°C and pH of 7. It did not retain activity with a 50°C 60 min-treatment; in contrast, it maintained 86% of its activity in NaCl 1.0 M (Fig. 2), and presented higher activity in absence of divalent cations (data not shown).

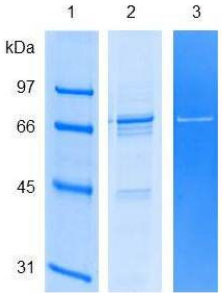

Fig.1 Expression of the recombinant protein. 1) Molecular weight marker, 2) Coomassie blue stained electrophoretic pattern, 3).zymogram against *M. lysodeikticus*. SDS-PAGE 10%

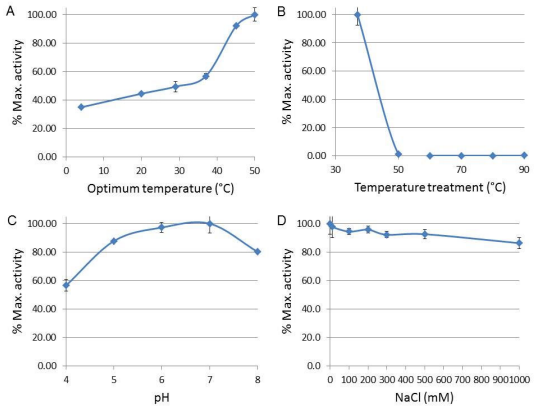

Fig.2 Characterization of the recombinant protein. A) Optimum temperature, B) thermal stability, C) optimum pH, D) effect of ionic strength on activity.

Conclusions. A novel PGH from *E. faecalis* was identified, cloned and characterized. Because of its inhibitory activity against pathogen microorganisms and catalytic properties, it has a potential use in food microbial safety.

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