INTRACELLULAR PROTEOLYTIC ACTIVITY OF *Pediococcus acidilactici* ATCC8042

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Introduction. Lactic acid bacteria (LAB) play a key role in fermented foods providing flavor, modifying their texture and participating in the preservation and ripening process [1]. The most studied LAB used as a starter culture in fermented foods is *Lactococcus lactis,* whose proteolytic system is already elucidated. Another LAB used as a starter culture, mostly in fermented meat products, is *Pediococcus acidilactici*. Its prototeolytic system hasn't been described yet [2] but genomic advances show an increasing number of genes encoding different proteases, these enzymes are broadly used in a variety of industries. Besides, the knowledge of the components of the proteolytic system in this starter would lead to the design of better suited strains used in the meat industry or to the production and application of these new catalysts in different fields.

Until now two proteolytic enzymes found in *Pediococcus acidilactici* have been characterized [4]. The aim of this project was to find enzymes in different culture conditions or cellular location and to characterize them, in order to better comprehend the proteolytic system of this LAB.

Methods. Cells from *P. acidilactici* were grown in 1 L of TSB medium at 29ºC. Samples were taken after the cultures were grown for 8, 12, 16 and 24 h, and optical density and pH were assayed. The cells were washed and the optical density was adjusted to 5. The pellet was sonicated to obtain the cytosol, which was then ultrafiltered and lyophilized. Proteolytic activity was determined by zymography with gelatin 1% as a substrate [3]. Casein, gelatin, collagen and elastin were assayed as substrates for the enzyme.

Results. The highest proteolytic activity was observed after 16 h of growth. The cytosolic fraction showed a proteolytic band active towards gelatin, approximately at 120 kDa, which was also observed in the SDS-PAGE 10%. Figure 1 shows that the activity was stable over a pH range 3-10, it was slightly diminished above 70°C, it was activated by 10 mM Ca^{2+} and inhibited with 10 mM PMSF.

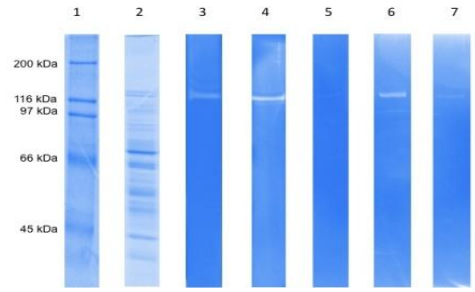

Figure 1 Cytosolic fraction SDS-PAGE 10% and zymograms against gelatin 1%. Lane 1, High range molecular marker; lane 2, electrophoretic profile; lane 3, zymogram with cytosol (control); lane 4, 10 mM Ca^{2+}; lane 5, 10 mM PMSF; lane 6, 60ºC 60 min; lane 7, 70ºC 60 min.

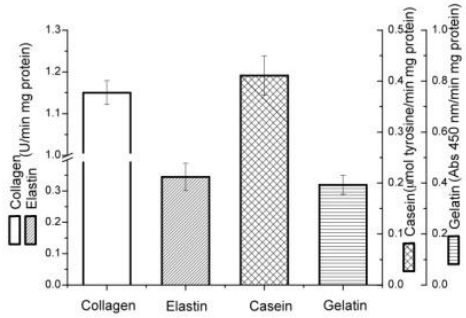

Figure 2. Substrate specificity of the cytosolic fraction. Collagen, elastin, casein and gelatin.

Figure 2 shows the enzyme is more active towards collagen or casein than to other proteins present in muscle or skeleton**.**

Conclusions. A new protease has been found in *P. acidilactici*, with a MW of 120 kDa, obtained from the cytosolic fraction when cultured for 16 h in TSB broth. Like the enzyme described previously by Casales (4) it is a serine protease, but both enzymes differ in their substrate preferences and are obtained in different culture conditions. Therefore, they must be physiologically and technologically complementary.

References.

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