ANTIBACTERIAL ACTIVITY OF *Pediococcus acidilactici* ATCC8042: PEPTIDOGLYCAN HYDROLASE

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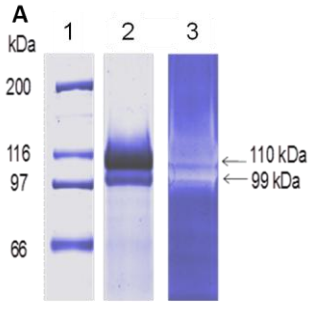
Introduction. Lactic acid bacteria are used as starter cultures to preserve food because they produce a wide variety of antibacterial metabolites as: acids, bacteriocins and peptidoglycan hydrolases (PGH) (1). These last enzymes hydrolyze glycosidic bonds or peptides found in the peptidoglycan, producing cell lysis, and are involved in several cell functions, such as growth, division, and autolysis (2). *P. acidilactici* ATCC 8042 produces intracellularly a lytic band of 116-kDa and two more lytic bands have been observed at 45- and 110-kDa, which are active against *Micrococcus lysodeikticus* (3). In addition, a lytic band at 116-kDa was recovered at very small amounts in cell supernatants (4).

The aim of this work was to find the growth phase and localization of the lytic activity, as well as to assess its effectiveness against pathogenic bacteria and to identify which of the above described proteins is responsible for the antibacterial activity

Methods. Proteins were extracted from cells grown in MRS medium collected at different growth stages. A three-step purification procedure involving membrane techniques was used. Mass spectrometry analysis (LC/ESI-MS/MS) was used to identify the purified proteins. The fraction containing the concentrated proteins (110- and 99-kDa) were evaluated against different pathogenic strains (table 1). The gene of the 99-kDa-protein was amplified from genomic DNA by PCR, cloned and expressed in *E. coli* BL21(DE3), using the pET-19b(+) as cloning vector and IPTG as inducer. Other cloning strategies are described elsewhere (5).

Results. The highest PGH activity was found during the logarithmic growth phase in the protein fraction bound to the cell membrane (data not shown).



B

Strains	mm mg prot.$^{-1}$
Gram positive	
Bacillus cereus CFQ-B-230	206.4
Bacillus subtilis ATCC 6633	127.2
Streptococcus pyogenes CFQ-B-218	188.9
Staphylococcus aureus ATCC 6538	180.2
Enterococcus faecium QB	99.4
Enterococcus faecalis QB	88.9
Listeria monocytogenes CFQ-B-103	77.8
Pediococcus acidilactici ATCC 8042	89.6
Lactobacillus paracasei CFQ-B-90	79.2
Gram negative	
Salmonella typhimurium ATCC 14028	200.6
Escherichia coli DH5a	76.6
Pseudomonas aeruginosa ATCC 27853	0.0

Fig. 1 A) Protein profile (SDS–PAGE, 10%) and zymogram of native proteins. Lane 1, high molecular weight marker; lane 2, partially purified proteins; lane 3, zymogram against *M. lysodeikticus*. **B)** Antibacterial spectrum.

The protein profile and zymogram shows two proteins with lytic activity (Fig. 1A) which inhibit the growth of several pathogenic strains (Fig. 1B). Mass spectrometry analysis (LC/ESI-MS/MS) indicated that the 110-kDa band corresponded to a protein of unknown function. The 99-kDa band corresponded to a *N*-acetylmuramidase that harbored catalytic sites with *N*-acetylmuramoyl-L-alanine amidase and *N*-acetylglucosaminidase activity. This protein was cloned and expressed in *E. coli* BL21 using pET-19b(+) as vector.

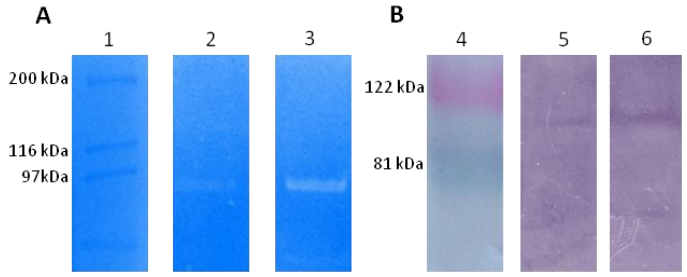


Fig. 2 A) Zymogram against *M. lysodeikticus*, **B)** Western blot of recombinant protein. Lane 1 and 4, high and kaleidoskope prestained molecular weight marker, respectively; lane 2 and 5, recombinant protein without induced; lane 3 and 6, recombinant protein with 1 mM IPTG.

Figure 2A shows the lytic activity against *M. lysodeikticus* of the recombinant protein, with a MW around 95 kDa. This activity is more intense in presence of inductor. Western blot evidence that the recombinant protein presents the histidine tail, which was detected by an antibody coupled to alkaline fosfatase (Fig. 2B).

Conclusions. *Pediococcus acidilactici* harbours at least two lytic enzymes. A protein of 110-kDa with previously unknown function is recognized as PGH for the first time, and exerts antibacterial activity against several bacterial strains. The 99-kDa protein was cloned and expressed in *E. coli*, and retained its lytic activity with a slightly inferior molecular weight (around of the 95-kDa). This recombinant protein has a potential use in food microbial safety as a new enzybiotic.

References.

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