MOLECULAR CLONING AND EXPRESION OF PEPTIDOGLYCAN HYDROLASE 99-kDa OF *Pediococcus acidilactici* ATCC 8042

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Introduction. Peptidoglycan hydrolases (PGH) are enzymes that act on glycosidic or peptidic bonds of the peptidoglycan present in bacterial cell walls. Their physiological role seems to be linked to cell death and fission. Recently, they have been used as food preservatives, as an alternative to antibiotics and to the development of hygienic surfaces [1, 2]. *P. acidilactici* ATCC 8042 has been shown to produce two lytic PGH proteins, with a MW of 110- and 99-kDa, which together exert a wide antimicrobial activity spectrum [3]. However, traditional purification methods have not yielded separate proteins due to their similar physicochemical properties [4].

Therefore, this work intends to obtain the 99-kDa protein by gene cloning and expression in *E. coli*. A gene described in the genome of *P. acidilactici* 7_4 [3] shows two conserved catalytic domains, a *N*-acetilglucosaminidase and a *N*-acetilmuramil-L-alanine amidase. Thus, the obtained recombinant protein may have the potential to be used as an antibacterial agent in food and related industries [2].

Methods. Specifically designed primers were used to amplify genomic DNA from *P. acidilactici* ATCC 8042 and obtain a 2800 pb amplicon. pET-22b(+) was used as cloning vector and the gene was expressed in *E. coli* XL 1-Blue®. Expression was achieved after testing different IPTG concentrations, incubation time and glucose as carbon source. Lytic activity was assayed in zymograms against *Micrococcus lysodeikticus* (0.2%w/v).

Results. Figure 1 shows the amplicons and digestion products after different molecular procedures.

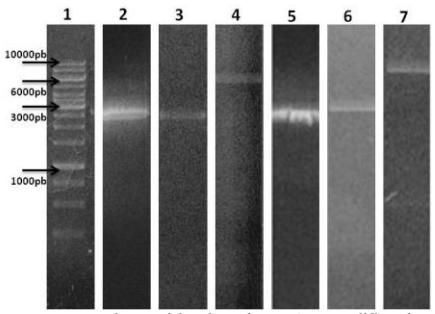

Figure 1. Lane 1, marker 1 kb plus; lane 2, amplification of target gene from DNA of *P. acidilactici*; lane 3, digest target gene; lane 4, digest pET22b(+) with Hind III; lane 5, PCR product with specifics primers; lane 6, PCR product with T7 primers; lane 7, digest whole construction pET22-99k

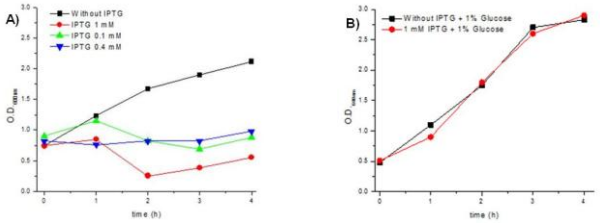

Figure 2 A) Effect of differents concentrations of IPTG and **B)** effect of glucose during induction of *E. coli* XL 1-Blue® pET22-99k.

Figure 2 shows the decay in optical density of the E. coli strain when different amounts of IPTG are used as inducer. The highest decay is exerted by 1 mM, which can be associated to the highest expression of the lytic protein and the lysis of the host cell. Figure 2B shows that glucose represses the expression of the recombinant lytic protein and therefore no decay in optical density is observed. The zymograms in Figure 3 indicate that a lytic band is expressed, but it has to be released through sonication and that the molecular weight is below the expected 99-kDa weight.

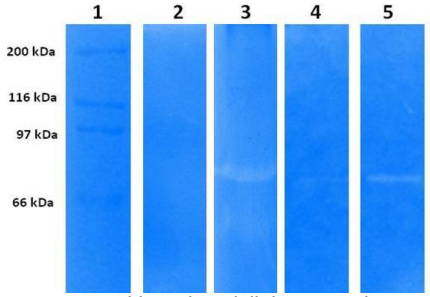

Figure 3. Zymogram with *M. lysodeikticus* as substrate. Lane 1, molecular weight marker; lane 2, whole cell proteins t=3 h; lane 4, whole cell proteins t=3 h + [IPTG] 1 mM; lane 4, sonicated cells t=3 h; lane 5, sonicated cells t=3 h + [IPTG] 1 mM.

Conclusion. The gene that encodes the 99-kDa PGH from *P. acidilactici* was cloned and expressed in *E. coli* when 1 mM de IPTG was used as inducer, 1% glucose was added to the medium and the cells were grown at 37°C for 3 h. The protein is active and lyses *M. lysodeikticus*, but the MW is only 72-kDa. Processing posttranslational conditions must be studied.

References

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