CARACTERIAZATION OF BACTERIOGENIC LACTIC ACID BACTERIA FOR MEAT BIOPRESERVATION

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Introduction. There is an increased interest in biopreservation through the use of lactic acid bacteria (LAB), because of their safe association with fermented foods. Bacteriogenic LAB produce antimicrobial peptides, known as bacteriocins, that can inhibit the growth of foodborne bacteria such as Listeria, Clostridium and Staphylococcus. Bacteriocins are a heterogeneous peptide group that varies in structure, biochemical properties, bactericidal spectrum and mode of action. These peptides are synthesized through a ribosomal pathway that involves transcription and translation mechanisms; genes that encode for their synthesis and autoimmunity are usually organized into groups of operons in the chromosome or in plasmids (1). The concern in the use of bacteriogenic LAB for food biopreservation, may be associated to the presence of virulence factors and the development of antimicrobial resistance.
The objective of this work is to present data related to the characterization of bacteriogenic LAB and their bacteriocins for their possible use in meat biopreservation.

Methods. Isolated bacteriogenic LAB were identified by 16S rDNA sequencing and BLAST homology search in the NCBI database. Strain antibiotics susceptibility was tested on Mueller-Hinton-agar by the disk diffusion method; the genomic DNA was used in PCR to detect genes encoding for virulence determinants; also the amino-decarboxylase activity was determined in a medium containing precursor amino acids. On the other hand, bacteriocins were purified by a modified adsorption-desorption pH dependent method and the amino acid sequence was obtained by automated N-terminal Edman-degradation. The inhibitory activity was based on their minimum inhibitory concentration (MIC) against several Listeria strains. The mode of action was evaluated by measuring the leakage of intracellular material and changes in the cell membrane morphology, as well as, the post-exposure recovery ability. In addition spontaneous bateriocin resistant strains were subjected to pulsed field gel electrophoresis (2, 3).

Results. Isolated bacteriogenic LAB were identified as *Enterococcus faecium*, *Enterococcus faecalis* and *Pediococcus acidilactici* with 100, 98 and 99.8% similarity, respectively. Among the virulence factors analyzed (*cylABM, agg, gelE, esp, cpd, cob, ccf, and ace*), the *ccf* gene, which encodes a sexual pheromone, was the only factor detected for the strains. *E. faecium* and *P. acidilactici* isolates were sensitive to all the tested antibiotics and demonstrated tyramine-decarboxylase activity. In contrast, *E. faecalis* was resistant to vancomycin and tetracycline, but did not produce biogenic amines.

Isolated bacteriocins showed the conserved N-terminal sequence YGNGV(X)C(X)4C (pediocin box'motif) demonstrating high similarity to other IIa pediocin-like bacteriocins (1). Regarding the inhibition of various species of Listeria, the growth rate decreased with increasing bacteriocin concentration. The three studied bacteriocins cause leakage of intracellular material; however, the one produced by *E. faecium* was the most effective, showing an inhibitory activity of 43.03 AU/mg of protein and a MIC of 7.81 mg/mL, achieving a reduction of Listeria in 1-2 log units. Also cell membrane damage was observed for treated strains (Fig 1).

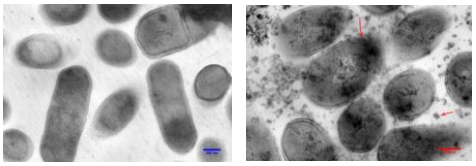

Fig.1 Transmission electron micrograph of *L. innocua* ATCC33090 (left-control cells; right: treated with the bacteriocin produced by *P. parvulus*).

Repetitive high bacteriocin exposure induced resistant *L. innocua* strain with changes in the PFGE genomic profiles (Fig.2), thus indicating that the resistance may be at genomic level. Although, resistant-type strains showed lower specific growth rate and lower glucose consumption with production of ethanol, formic and acetic acids, in comparison with the wild-type strains that produced mixed acid pathway.

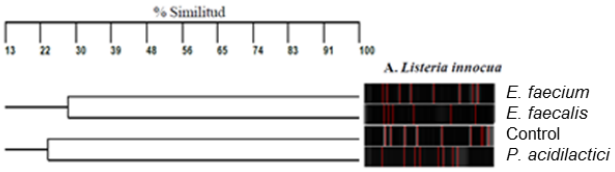

Fig. 2 PFGE genomic profiles of resistant *L. innocua* strains induced by repetitive exposition to bacteriocins.

Conclusions. Bacteriogenic isolates demonstrated their inhibition action, but it is necessary to perform further studies to identify the mechanism of resistance generation when used at high levels.

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References.

1. Cotter P, Hill C, Ross R. (2005) *Nat Microbiol Rev*. 3:777-788.
2. Álvarez-Cisneros Y, Fernández F, Wacher C, Aguilar M, Sainz T, Ponce-Alquicira E. (2010) *J Sci Food Agric*. 90: 2475–2481.
3. Regininni L, De-Souza A, Brandelli A. (2008*) J. Microbiol*. 48: 393-400.