ANTITUMOR EFFECT OF HPV-16 E7 ANTIGEN FUSED TO EXOTOXIN A TRANSLOCATION DOMAIN SECRETED BY LACTOCOCCUS LACTIS.

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Introduction. Cervical cancer results from the infection of Human Papillomavirus. HPV type 16 is known to cause at least 58.9% of al cases of cervical cancer due to this infection. The HPV E7 viral gene encodes for a protein of the same name that acts as an oncogenic protein (1). Developing strategies to protect against this protein is essential to prevent the transformation of HPV-infected cells. The bacterium *Lactococcus lactis* is a Gram-positive organism, not sporulated, not mobile and food grade. It has recently been used to express heterologous proteins of medical interest due to its innocuous characteristics (2). The TC-1 cell line was derived from lung epithelial cells of C57BL/6 mice. These cells are positive for expression of the E6 and E7 protein from HPV-16. These features make the TC-1 model helpful to test specific immunotherapeutic strategies against E6/E7. The fragment IA-IB-II of exotoxin A from *P. aeruginosa* has been used for the intracellular delivery of some antigens with promising results (3,4).
In this study we created a *L. lactis* strain that secretes a fusion protein conformed by the ExoA toxin with the E7 antigen and it was tested in a murine tumor model to evaluate the protection of this recombinant strain.

Methodology. To evaluate the anti-tumoral activity of the pSECExoA-E7 strain of *L.lactis* we used a cervical cancer mouse model. The ExoA (dIII)-E7-KDEL gene used in this work was synthetized by GENEART. This gene was inserted in the plasmid entitled pSecExoE7 which has a nisin-inducible promotor, and a chloramphenicol resistance gene. By electroporation, we transformed this plasmid in wild-type strain of *L.lactis*, NZ9000. Western Blot using monoclonal antibodies against Exotoxin A and E7 confirmed expression and secretion of the fusion protein. Once the recombinant strains were obtained, we performed an antitumoral assay with 6 mice per group immunized with the following treatments: NZ900 as negative control, pSECE7 that secretes E7wt, E7/CWA that expresses E7 on its cell wall, and ExoE7. Mice were immunized on days 0, 15, 30 and 35. On day 40 of the immunization schedule the mice were challenged with the cell line TC-1 that expresses the antigen E7 of HPV-16.

Results and Discussion. In figure 1 we show the schematic design for Exo-E7 gene and a characterization with restriction enzymes that liberates a band of 600pb as expected. Figure 2 shows the expression of the protein ExoAE7 in *L. lactis* demonstrated in two clones by western blot using specific Anti-ExoA antibodies. Furthermore we found an important antitumoral effect in the group immunized with Exo-E7 compared to Nz9000 control group.

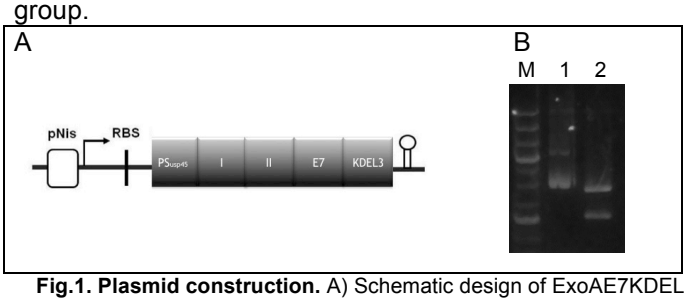

Fig.1. Plasmid construction. A) Schematic design of ExoAE7KDEL expression, B) Characterization of plasmid DNA with BamHI and NotI resctriction enzymes, Lines: M-molecular ladder, 1-Plasmid 2-Plasmid Digestion.

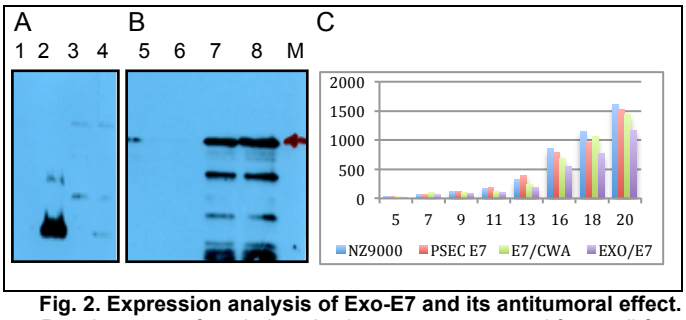

Fig. 2. Expression analysis of Exo-E7 and its antitumoral effect. Protein extracts from induced cultures were prepare-d from cell-free samples and analyzed by Western blot. A) Incubated with antibody against E7. Lines: 1) NZ9000, 2) pSecE7, 3) pSecExoAE7, 4) pSecExoAE7, B) Antibody against ExoA. Lines: 5) NZ9000, 6) pSecE7, 7) pSecExoAE7, 8) pSecExoAE7, M) protein molecular marker. C) Antitumoral effect of Exo-E7.

Conclusions. *Lactococcus lactis* is capable to induce a cellular immune response by secreting a tumor antigen fused to exotoxin A of *Pseudomonas aeruginosa*.

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