PRODUCTION OF HUMAN INTERLEUKIN-22 IN LACTOCOCCUS LACTIS

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Introduction. Human Interleukin 22 (IL-22) belongs to a group of IL-10 related proteins also known as Interleukin 10-related T cell-derived inducible factor (IL-TIF). IL-22 was identified for the first time as a gene induced by IL-9 in mouse T cells. Human IL-22 cytokine has 25% homology to IL-10, nevertheless, they are functionally different. IL-22 acts mainly on skin cells, digestive and respiratory systems and is primarily associated with the maintenance of barrier function and induction of innate antimicrobial molecules at mucosal surfaces (1). It has been shown to drive the production of many antimicrobial peptides, including β-defensins, S100-family proteins, and regenerating-gene (Reg)-family proteins and by now a variety of IL-22 mediated functions has been reported in different settings such as cancer, infectious and inflammatory diseases (2). The gram-positive and non-pathogenic lactic acid bacteria (LAB) are considered promising candidates for the development of oral live vaccines. *Lactococcus lactis* is of particular interest for oral delivery of functional proteins since it is a non-commensal food bacterium that is incapable to survive in the digestive tracts of animal models and humans (2).
In this work we obtained a recombinant strain of *L. lactis* capable to secrete human IL-22 recombinant protein using a nisin-controlled expression system.

Methodology. Human IL-22 gene used in this work was synthetized by GeneArt Company. It was codon optimized for efficient expression in *L. lactis*, and its signal peptide was substituted for the usp45 signal peptide that is found in a native secretion protein from this strain. The sequence was flanqued with *BamH*I and *Not*I restriction sites that served to clone this sequence into the PVE3684 plasmid. This vector was named pSECIL22 and has the nisin inducible promotor, Pnis, a chloramphenicol resistance gene, CmR, and the ribosome binding site, RBS. This expression vector was introduced by electroporation into the *L. lactis* strain NZ9000 that has the nisRK genes on its chromosome. Three chloramphenicol resistant clones were characterized by restriction enzymes and the production of the protein of interest was confirmed by Western blot with monoclonal antibodies. In order to perform a quantification of protein secretion we collected supernatants after different induction times and analysed them by an ELISA test following the manufacturer's instructions.

Results and Discussion. In figure 1A we show the plasmids used for IL-22 construction. Expression of IL-22 protein in *L. lactis* was demonstrated in three clones by western blot using specific antibodies. As show in figure 2 all clones were capable of releasing IL-22 protein in the expected size as compared to commercial recombinant protein loaded on R line. Furthermore, we quantified the protein secretion by an ELISA test and we found that the maximal expression is shown after 4 hours induction with a concentration of 4 ng/ml.

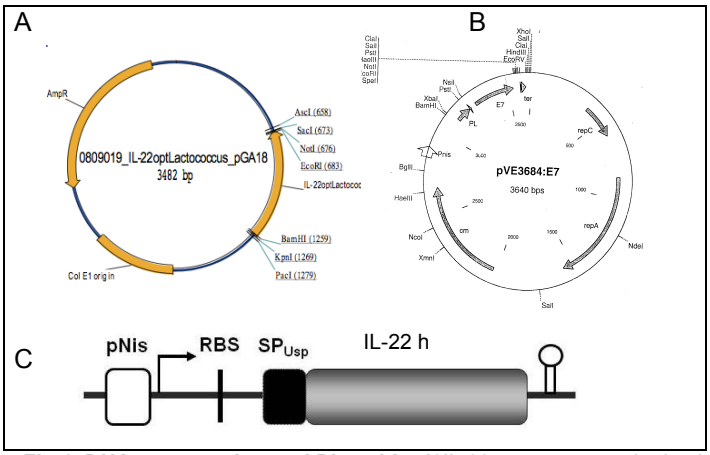

Fig.1. DNA construction and Plasmids. *A)IL-22 gene was synthetized and sub-cloned in pGA18 plasmid. B)It was flanqued by BamHI and NotI sites that were used to clone it into pVE364. C)* Schematic design of IL-22 expression system for production and secretion by *Lactococcus lactis*.

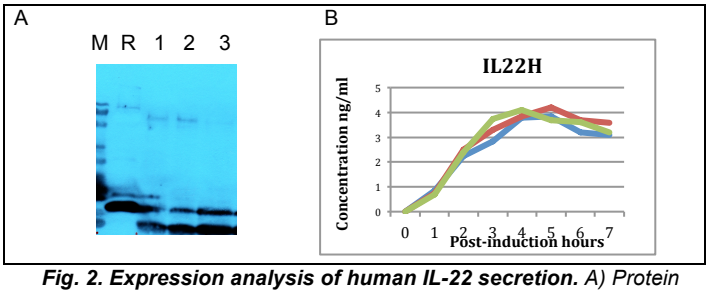

Fig. 2. Expression analysis of human IL-22 secretion. *A) Protein extracts from induced cultures were prepared from cell-free samples and analyzed by Western blot. M, protein molecular marker. R, commercial recombinant protein. B) ELISA test from induced cultures in through seven hours after nisin-induction.*

Conclusions. In this work we obtained a *L. lactis* recombinant strain that produces human IL-22 cytokine by a nisin-induced controlled system.

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