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STUDY OF rpoH GENE EXPRESSION IN *Xanthomonas axonopodis pv. citri.*

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High temperatures and other environmental factors of stress in such a way induce the expression of some heat shock proteins in prokaryotes and eukaryotes. These molecular proteins include chaperones, as well as some proteases as Lon and Clp, acting in the degradation of denatured proteins. The control of the heat shock proteins expression occurs at transcriptional level under control of the rpoH (sigma 32) gene product allowing the RNA polimerase recognizes specifically HSG (Heat Shock Genes) promoters. Cancro citric, illness caused by *Xanthomonas axonopodis pv.citri*, gram-negative bacteria, which attack different hosts of economic importance including citrus, rice, beans, grape and cotton, and since the bacteria need to survive in the host exposed to a large range of temperature, between 15 and 50 degrees Celsius approximately, it is a great deal to study the presence of HSG which helps *Xanthomonas* installation and multiplication in different organs of the plant.

This work has been done in order to analyze rpoH gene expression in *Xanthomonas axonopodis pv. citri.* Using bioinformatic tools we were able to select clones that contain the full-length sequence of this gene. After sequencing to confirm the selected clones they were used to transform sigma 32-deficient *E. coli.* Two of five clones analyzed showed functionality, meaning that bacteria survive at higher temperatures only when the whole rpoH gene was present.

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Synthesis of the *Schistosoma mansoni* sm14 Gene with Mycobacterial Codon Usage for Expression in Recombinant BCG.

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Recent research has shown that an A +T rich genome may offer some restrictions for expression in heterologous systems, because numerous cryptic polyadenilation sites are present. A gene with codon usage compatible with the specific system can be better expressed. The polymerase chain reaction (PCR) can be used to assemble and amplify oligonucleotides to produce a synthetic gene, which can then be cloned and expressed in an heterologous system.

First, the native sm14 gene of *Schistossoma mansoni* was cloned and expressed in BCG under control of the mutated β-lactamase promoter, pBlaF*. Although we observed low expression of the antigen in BCG, it was enough to induce a cellular response in immunized mice with IFN-γ production. We then used oligonucleotide assembly PCR to synthetize an sm14 gene with the codon usage for mycobacteria. Mutations were corrected by complementary PCR. The PCR product, was cloned, sequenced and will be inserted into different mycobacterial expression vectors to compare the expression with that obtained by native sm14 in BCG. Immune response induction by these recombinant BCG-Sm14 vaccines against helminths will be evaluated.

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APPROACHES TO MAXIMIZING THE PERIPLASMIC EXPRESSION IN *ESCHERICHIA COLI* OF THE HUMAN GROWTH HORMONE GENE FROM A VECTOR CONTAINING THE λP_L PROMOTER

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In this work we have investigated the influence of different factors acting on the periplasmic expression of recombinant human growth hormone (hGH) in *E. coli.* A bacterial vector containing the phage lambda P_L promoter, which is activated by the temperature, was utilized. Four different signal peptides were tested: DsbA, npr, STII and one derived from the natural hGH signal peptide. Other factors such as medium, optimized induction and, expression conditions, and different bacterial strains were also studied.

The determination of hGH directly in osmotic shock fluids was based in an isocratic reversed-phase high-performance liquid chromatography (RP-HPLC) method. This methodology allows an initial rapid evaluation of the quality and quantity of hGH being secreted in the bacterial periplasmic space right after, or even during fermentation.

The level of hGH production increased approximately 100% compared to the reference vector reaching a level of 3.0 ± 0.3 μg/mL/A_{600}.(n = 3 - CV = 9.0%). The expression level was largely affected by the induction conditions showing to be more effective in the lately logarithmic phase. Our results indicated that 6h activation at 42 °C starting with na on O.D. of ~3 for a rich medium were essential in order to get the maximum expression level. He rich medium, compared to LB medium, showed a better hGH production per liter of medium.

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Disruption of the beta-Lactamase Gene in *M.bovis* BCG: Investigation of its Resistance to Penicillins and Cephalosporins

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The genus *Mycobacterium* includes a number of pathogens that cause tuberculosis (*M. tuberculosis, M.bovis*), leprosy (*M.leprae*) and diseases affecting immunocompromised subjects (*M.avium*). The treatment of these diseases is difficult because mycobacteria are naturally resistant to a great variety of antibiotics. The aim of this project is to obtain a *M.bovis* BCG strain without resistance to beta-lactamic compounds (penicillins and cephalosporins), by the disruption of the beta-lactamase gene. Previously, the gene was obtained using PCR, disrupted and cloned into a plasmid containing *hyg* and *sacB* genes, for double selection of the transformants.

Transformants from counter selection in hygromicin and sucrose were obtained. Genomic DNA was extracted from twenty of the these clones and confirmation of recombination was perfomed by PCR using specific primers for the beta-lactamase gene. At least one of the clones amplified the beta-lactamase gene with reduced size.

Alterations due to homologous recombination are being evaluated, such as the new MIC for the beta-lactams, kinetic parameters and persistence of the new strain in mice. If the lower resistance to antibiotics is confirmed in this new stain, it can be used for vaccination of immunocompromised individuals and the beta-lactamase locus can be used as an insertion site for the expression of heterologous antigens.

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DNA vaccines against *Streptococcus pneumoniae* based on PspA (pneumococcal surface protein A)

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Streptococcus pneumoniae is a major cause of disease, including pneumonia, otitis, meningitis and bacteremia. About 1 million children die of pneumococcal disease every year, mostly in developing countries, but elderly persons are also at risk. Cheaper alternatives for the currently licensed vaccines are thus needed. We have constructed DNA vaccines based on PspA (pneumococcal surface protein A), which was shown to induce protection against pneumococcal bacteremia. PspA shows significant antigenic diversity, being divided into 3 different families and subdivided into 6 clades. Since most clinical isolates have PspA belonging to family 1 or family 2, PspA fragments from both families were analyzed. DNA vaccines encoding the complete N-terminal region of PspA (clade 1 or clade 3) elicited significant humoral response and cross-reactivity was mainly restricted to the same family of PspA. DNA vaccines encoding fusions between PspA fragments from family 1 and family 2 (clade 1-4 or clade 3-2) were also constructed and were able to broaden cross-reactivity, with the induction of antibodies that show reaction with both families of PspA. Challenge experiments will be performed in order to analyze if these DNA vaccine vectors are indeed able to induce cross-protection against *S. pneumoniae.*

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A PLANTIBODY AGAINST BREAST CANCER

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Among the new strategies to find a good therapy for malignant tumors, the generation of specific antibodies for cancer antigens has been viewed as one of the most promising. Attempting to optimize this approach, some research has been conducted to create molecules that are not whole immunoglobullins, but only the variable portion of the Igs. These molecules are called scFv antibodies. Plants have been increasingly considered as cost efficient and contamination safe factories for the production of recombinant mammalian proteins. Once antibodies to fight cancer are often very expensive, these molecules may be one of the most interesting types of recombinant proteins to be produced by this technology. Studies with breast cancer made in the Laboratorio de Oncología Básica - Facultad de Medicina, Montevideo, Uruguay, had led to the use of cell suspensions (obtained from cancerous tissue sections of patients with this kind of tumor) to immunize rats in order to generate antibodies candidates to be used in immunotherapy and diagnosis. Using this strategy, one monoclonal antibody was produced from the 83D4 hybridoma. The aim of this work is the use of transgenic tobacco seeds as a system for the production of correctly folded and glycosilated scFv 83D4 molecules. The transgenic plants were obtained by the *Agrobacterium tumefaciens* mediated transformation of leaf discs. The T-DNA contains the 83D4 scFv coding sequence under the control of an endosperm specific promoter and the 35S terminator. Southern blotting analysis confirmed the integration of the gene of interest in the genome of most of the kanamycin-selected plants. Western blotting of protein extracts of the transgenic plants revealed that the 83D4 scFv antibody is being expressed in seeds in considerable amounts. We are now making efforts to purify this molecule from seed extracts to evaluate its specificity by ELISA and immunohistochemistry and its glycosilation pattern using mass spectrometry.

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