

## **PRODUCTION OF RECOMBINANT STREPTOLYSIN-O FOR DIAGNOSTIC USE**

**Thesis:** B. Velázquez submitted this thesis for her Masters in Biotechnology at the Post-Graduation Program, Faculty of Sciences, Universidad de la República, Montevideo, Uruguay, 2005.

**Advisors:** Professor Hugo Massaldi and Professor Alejandro Chabalgoity

**ABSTRACT:** The current thesis describes the production of a recombinant form of streptolysin-O, appropriate for use in immunoassays, as well as the optimization of the production process in a laboratory fermenter. Determination of anti-streptolysin-O antibody titers is one of the paraclinical assays most often used in the diagnosis of sequelae to *Streptococcus-pyogenes* infections. To develop diagnostic reagents, it is necessary to obtain streptolysin-O in suitable quantity and quality. Producing streptolysin-O from *S. pyogenes* cultures is difficult since it requires complex recovery and purification protocols and the yield is low; therefore, large volumes of bacterial culture must be handled. Because of these difficulties, an alternative approach was considered: using recombinant DNA techniques to produce recombinant streptolysin-O protein. This approach allows high yields of proteins using relatively simple purification protocols in better biosafety conditions. This thesis reports the cloning of the streptolysin-O gene from nucleotide 430 to the end of the reading frame, in the expression vector pGEX-2T. Recombinant streptolysin-O protein was expressed in *Escherichia coli* as a fusion protein with the glutathione S-transferase enzyme of *Schistosoma japonicum*. In order to increase production of the recombinant protein in *E. coli*, its expression was studied in cultures in small flasks, analyzing variables such as inducer concentration, induction temperature, proportion of culture medium volume to total flask volume, and different *E. coli* strains. In a second stage, production was scaled up to fermenter level and optimized; the volumetric rates of oxygen consumption and glucose feeding of the system were analyzed. After cultivation in the fermenter, the recombinant streptolysin-O was recovered and purified by affinity chromatography on a glutathione-Sepharose column. Finally, the stability of the final product in different formulations was

analyzed. Stability was characterized by the antigenicity and the hemolytic activity of the protein product after storage. Based on the optimization studies, a protocol was defined for the production of recombinant streptolysin-O under controlled conditions of temperature, pH and glucose supply, with variation in the volumetric rate of oxygen consumption: high during the growth phase and low during the induction phase. Immunoblot assays carried out using the recombinant protein demonstrated that it was specifically recognized by human sera positive for anti-streptolysin-O antibodies. An ELISA was therefore developed to measure anti-streptolysin-O antibody titers in human sera and was compared with a commercial latex kit which used native streptolysin-O as the antigen. Results indicated that the recombinant streptolysin-O was suitable for use in immunoassays. In summary, the results showed that a simple and safe protocol was developed for the production of recombinant streptolysin-O suitable for use in immunoassays, with a yield approximately 30-fold higher than that obtained in traditional production systems using *S. pyogenes* cultures. This protocol could be used as the basis for developing industrial scale production of recombinant streptolysin-O and mass production of diagnostic kits for *S. pyogenes* infections and their sequelae.

**KEY WORDS:** streptolysin-O production, recombinant proteins, *Escherichia coli*, fermenter, optimization culture.

**CORRESPONDENCE TO:**

BLANCA VELÁZQUEZ, Department of Biotechnological Development, Institute of Hygiene, Faculty of Medicine, Universidad de la República. Av. Alfredo Navarro 3051, 11600, Montevideo, Uruguay. Phone: + (598 2) 487 12 88 ext. 1120. Fax: + (598 2) 487 30 73. Email: [bvela@higiene.edu.uy](mailto:bvela@higiene.edu.uy).