BIO_26 - Development of an expression platform in Escherichia coli for the production of anakinra biosimilar

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Introduction: Different prokaryotic expression systems are used to produce biopharmaceuticals, especially *E. coli*, a well-characterized species with low costs, high productivity, fast production cycles and ideal for producing small proteins that don't need post-translational modifications. The extracellular secretion of these biomolecules in *E. coli* culture media offers significant advantages over intracellular strategies. The main advantage is the simplified recovery and purification process, which significantly reduces production costs. In addition, extracellular secretion can prevent the target protein from accumulating in the cytosol as inclusion bodies, increasing the stability and biological activity of this macromolecule. Efficient methods that allow the translocation of biomolecules beyond the outer membrane barrier are still poorly available and challenging on an industrial scale.

Objectives: The aim of this research project is to develop national platforms for the production of the biopharmaceutical anakinra (interleukin 1 receptor antagonist) and the enzyme enterokinase in *E. coli* strains, with a view to the future development of biosimilars.

Methodology: To carry out this work, pET-29(+) plasmids were made with the sequences of interest and inoculated by bacterial transformation into *E. coli* BL21 (DE3) strains. After the initial analysis of the heterologous expression test, new tests were carried out in a shaker incubator to assess the best time, temperature, culture medium and culture supplementation.

Results: Using an export protein coupled with a signal peptide and the sequence of amino acid residues of the therapeutic protein, positive expression and export to the periplasm were achieved using defined and undefined autoinducing media at 18 and 37°C, respectively, for 48 hours, with demonstration on SDS-PAGE and Western Blotting gels. Subsequent steps such as purification using affinity chromatography were successful in obtaining anakinra. Mass spectrometry analysis will be used to identify the protein.

Conclusion: The results of these analyses are decisive for the follow-up of the project in *in vitro* and *in vivo* tests, expansion of production processes in bioreactors and formulation development.

Keywords: Therapeutic proteins; Biopharmaceuticals; Biosimilars