BIO_07 - Upstream optimization of recombinant L-asparaginase production in *E. coli* for the treatment of Acute Lymphoblastic Leukemia

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Introduction: Acute Lymphoblastic Leukemia is a neoplastic disease that mainly affects children. Initial treatment includes biological medications such as L-asparaginase, an enzyme that acts on the degradation of asparagine, essential for grow and proliferation of leukemic cells. Treatment with L-asparaginase may result in allergic and toxicity reactions due to its bacterial origin. Alternatively, our group is working on the production of human L-asparaginase (hASRGL1) recombinantly in *E. coli*. Structural studies have shown that replacing glycine (G) by glutamate (E) at position 10 results in greater enzymatic activity, favoring treatment efficacy. To enable the production of hASRGL1_G10E, it is necessary to optimize the production process, seeking the parameters that most impact enzyme production.

Objectives: The aim of this work is to optimize the production of recombinant human L-asparaginase in soluble and active form applying design of experiment (DOE).

Methodology: Recombinant *E. coli* were grown in shaker flasks using the parameters stablished by Plackett– Burman design (Protimiza). Assays were grouped into two parts: a kinetic test, that would determine best induction time, and another for the remaining parameters, such as media composition, pre-cultivation time, temperature and inductor concentration. Results were analyzed by SDS PAGE and western blot with ARSGL1- specific antibody.

Results: Kinetic assays showed that protein expression did not change upon increasing the time of induction. Best productivity was achieved after 8 hours of induction. Based on this, following experiments were conducted with 8 hours of induction. All parameters were tested at induction temperature of 18°C and we are currently running assays at 37°C. For assays, protein expression was verified by SDS PAGE and western blotting comparing culture samples before and after induction. An overlap of E. coli proteins and ASRGL1_G10E bands was observed, but western blot analyses confirmed the presence of the enzyme.

Conclusion: The results obtained so far indicate that the best induction time to produce L-asparaginase is 8 hours. New studies will be conducted targeting the expression of soluble L-asparaginase at 37°C in order to statistically evaluate how the parameters influence enzyme expression.

Keywords: L-asparaginase; Design of experiment; Protein expression