BIO.15 - Characterization of enzymatic activity and evaluation of cytotoxic effect of a *Bacillus subtilis* L-asparaginase type II expressed in *Escherichia coli*

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Introduction: Acute Lymphoid Leukemia is a cancer of blood cells, specifically lymphocytes, in which leukemic lymphoblasts require free L-asparagine in plasma to proliferate. L-asparaginase is a therapeutic enzyme that hydrolyses this amino acid, depleting its serum levels and, consequently, inhibiting the proliferative potential of cells tumors. Currently, the main formulations of asparaginase available are from *Escherichia coli* and *Erwinia chrysanthemi*, however there are reports of hypersensitive reactions in many patients, probably associated with the immunogenicity of enzyme. Therefore, it is important to search for other sources of L-asparaginase II, which could present fewer side effects, in addition to obtaining a national production of this biopharmaceutical.

Objective: Characterize a recombinant L-asparaginase II of *Bacillus subtilis* expressed heterologously in *E. coli,* determining the influence of pH and temperature on its enzymatic activity, its kinetic parameters and evaluate its cytotoxic effect in leukemic lymphoblastic cell line.

Methodology: The ansZ gene from *B. subtilis*, which encodes an asparaginase II, was cloned in pET28a vector and the heterologous expression in *E. coli* Rosetta occurred for 4 hours at 30ºC, using IPTG (0.5 mM) as inducer. Purification was performed using affinity chromatography with immobilized nickel, and recombinant enzyme was eluted with buffer contain high concentration of imidazole (250 mM). A colorimetric method based on Nessler reagent was used for evaluation of enzymatic activity under different pHs (3.0 to 11.0) and temperatures (20º and 90ºC), as well as for determination of kinetic parameters. AlamarBlue® cytotoxicity assay was performed with concentration ranges between 5 and 80 ug/mL of enzyme for 48 hours using culture of Raji cells.

Results: Heterologous L-asparaginase II of B. subtilis was expressed in soluble form and showed maximum enzymatic activity at pH 7.0 and temperature of 50°C. Saturation of its active sites was achieved with 4 μmol of L-asparagine, attaining Km of 1.427 mM and Vmax of 176.1 μmol/min/mg. About cytotoxicity, the treatment of Raji cells with B. subtilis L-asparaginase II showed considerable cell death only at the highest concentrations tested (70 and 80 μg/mL).

Conclusion: The results indicated that the recombinant L-asparaginase II of B. subtilis showed maximum activity bordering at physiological pH, and optimum temperature of 50° C. When compared the kinetics values to the corresponding enzyme from *E. coli*, the Km of L-asparaginase from *B. subtilis* is highest, that means lower affinity for substrate. This result complements the findings on AlamarBlue® assays, in which only high concentrations of recombinant enzyme presented cytotoxic action. Theses results open perspectives for protein engineering studies, aiming to increase enzymatic affinity as well as its cytotoxicity.

Keywords: L-asparaginase; Acute Lymphoid Leukemia; *Bacillus subtilis*