**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 30°C, 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 (20° and 90°C), 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*