Production and characterization of recombinant *Aliivibrio fischeri* L-Asparaginase with low L-Glutamine affinity: a potential antileukemic drug obtained by genetic engineering

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ABSTRACT

L-Asparaginase has been successfully applied in the treatment of lymphoid malignancies. Some limitations in the use of the commercial preparations of this drug include several side effects that may be correlated to L-Glutaminase activity, as immunosuppressive effects (Castro et al., 2021). The objective of this study was to evaluate the characteristics of a novel engineered *Aliivibrio fischeri* L-asparaginase type II expressed by *Bacillus subtilis*. Cultivations were carried out in shaken flasks at 30 ºC, 200 rpm, 24 h, using Luria-Bertani medium. Intracellular enzyme was recovered by sonication and enzymatic activities were evaluated by Nessler colorimetric method (Mashburn; Wriston, 1963). Recovered enzymatic extracts achieved L-Asparaginase activity up to 1.43 $U.mL^{-1}$ at optimum pH 7.5. Substrate affinity was much higher for L-Asparagine than for L-Glutamine (K_m= 1.226 $mmol.L^{-1}$ and K_m= 28.584 $mmol.L^{-1}$, respectively), which indicate the potential application of the recombinant enzyme as biopharmaceutical.

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