Title: OPTIMIZATION OF L-ASPARAGINASE PRODUCTION OF *STREPTOMYCES ANSOCHROMOGENES* TUR 10

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Abstract: Streptomyces, one actinobacteria gender, have been studied more each day for being characterized as a major producer of a variety of secondary metabolites, including enzymes such as L-asparaginase. This enzyme hydrolyzes asparagine and releasing ammonia yielding aspartyl enzyme. This intermediate reacts with water to form aspartic acid. Due to this action, L-asparaginase is an important chemotherapeutic agent in the treatment of acute lymphoblastic leukemia (ALL), Hodgkin's disease, tumors NK, T cell lymphomas and some subtypes of myeloid leukemias. The objective of this study was to evaluate the production of L-asparaginase by *Streptomyces ansochromogenes* TUR 10 and optimize the production conditions of this enzyme. The qualitative assay was performed using the standardized inoculum of actinobacteria in media CZ and M9 with the addition of phenol red as an indicator and L-asparagine as substrates. Quantification of L-asparaginase enzyme was determined by analysis of parameters such as: carbon sources (M9 media and TGY), fermentation time (24 to 120 hours), pH (5 to 9) and temperature (25 °C to 50 °C). The enzyme activity peak of each individual standards allow optimization of enzyme production conditions. The enzyme activity was determined by measuring the amount of ammonia formed by nesslerization, where 1 U of L-asparaginase was equal to the amount of enzyme which released 1 μM of ammonia per minute at 37 °C. The qualitative assay showed the production of the enzyme through a pink halo around the colony. Through the quantitative assay was determined that the M9 medium was best suited for production of the enzyme, with 133.9 U/mL, 3 times greater value than in the TGY medium. The kinetics showed a peak enzyme production in 48 hour period with values reached 197.27 U/mL. The pH of 6 was considered optimal for the production of L-asparaginase as compared to other pH, having an amount of 743.29 U/mL. The temperature 35 °C corresponding to more favorable condition for enzyme production among all analyzed temperatures, reaching 382.2 U/mL. The optimized conditions allow this way, the large-scale production to be successful, with levels of L-asparaginase quite high. This study demonstrates the potential of actinomycetes, especially the genus Streptomyces, such as large producers of L-asparaginase enzyme. It is essential to use different sources producing this enzyme, once L-asparaginase is a chemotherapeutic potential used for the treatment of leukemias.

Keywords: Asparaginase; Actinobacteria; Enzyme activity.

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