Title: PH AND THERMAL STABILITIES OF EXTRACELLULAR L-ASPARAGINASE PRODUCED FROM *Aspergillus terreus*

Authors: Torres, K.M.[1], Lopes, A.M.[1], Costa-Silva, T.A. [1], Pessoa-Jr, A.[1], Farias, J.G.[2]

Institution:
[1]Department of Biochemical and Pharmaceutical Technology (FCF/USP), School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brasil.
[2]Departament of Chemical Engineering, Faculty of Engineering and Science, University of La Frontera, Temuco, Chile.

Abstract*:*

The L-asparaginase (L-ASNase) catalyzes the hydrolysis of L-aspargine into L-aspartic acid and ammonia. This effect results in cytotoxicity for leukemic cell, and for that reason L-ASNase has been a clinically acceptable anti-tumour agent for the effective treatment of acute lymphoblastic leukemia (ALL). L-ASNase production using microbial system had attracted considerable attention, owing to the cost effective and eco-friendly nature. A wide range of microorganisms such as filamentous fungi have proved to be the good sources of L-ASNase. *Aspergillus terreus* (PC-1.7.A) under certain conditions can produce high amounts of extracellular L-ASNase, which is greatly favor in the enzyme production and downstream processes. Our work aims to study the enzymatic stability at different pHs and temperatures conditions of L-ASNase from *A. terreus*. The *A. terreus* strain was grown on potato dextrose agar (PDA) solid medium for 7 days at 30°C. For the pre-inoculum, 10^7 spores/mL were inoculated and was cultivated 17 h, 120 rpm at 30°C in modified Czaped Dox medium (pH 8.5) with 2% L-proline as nitrogen source. The inoculum was grown for 96 h at the same condition, but on medium without inorganic nitrogen sources. The culture was centrifuged and filtrated through a 0.22 µm membrane. The filtrate was incubated at 40°C in different pH used McIlvaine buffer for pH 3.0, 5.0, 7.0 and 7.5 and 50 mM Tris-HCl buffer for pH 9.0, 9.5 and 11.0. The optimal pH was used for thermic stability at 10, 20, 30, 37, 40 and 50°C. Assays measure time was 0, 3, 9 and 24 h. Total protein concentration was determined by the Bradford method and enzymatic activity determinations by the Nessler method. The highest specific activity (4.4 U/mg) was obtained under the conditions of pH 7.5 and 40°C, similar values at the physiological condition where L-ASNase acting. Acids pH reduced activity in more than 50% (pH 3.0 in 9 h), while alkaline pH maintaining high activity levels on different time periods studied (4.3 U/mg and 4.1 U/mg in 9 h at pH 9.0 and 9.5 respectively). According to the results, L-ASNase is extremely sensitive to slight changes in temperature (especially at temperatures of 10, 20, 30 and 50 °C), however the enzyme maintained 80% of its maximum activity (3.2 U/mg) at physiological temperature (37°C). Altogether, these data prompted further investigations into the extracellular L-ASNase produced by *A. terreus* (PC-1.7.A).

Key words: L-Asparaginase, *Aspergillus terreus*, enzymatic activity, enzymatic stability.
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