TITLE: SCREENING OF FILAMENTOUS FUNGI FOR PRODUCTION OF L-ASPARAGINASE

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ABSTRACT:

L-asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) is an enzyme widely used in the treatment of leukemias, mainly acute lymphoid leukemia (ALL), hydrolyzing asparagine, an important amino acid for tumor cell growth, in aspartic acid and ammonia. However, the commercially L-asparaginase available comes from bacteria, and causes various side effects in patients, culminating in drug resistance and loss of treatment efficacy. Therefore, the aim of this study was searching of filamentous fungi able to produce L-asparaginase. Twelve filamentous fungi (*Aspergillus labruscus, Aspergillus phoenicis, Aspergillus caespitosus, Aspergillus niveus, Aspergillus aculeatus, Aspergillus tamarii, Aspergillus parasiticus, Aspergillus thermomutatus, Paecilomyces variotii, Fusarium lateritium, Rhizopus microsporus* var. *microsporus* and *Metharizium anisopliae*) were submitted to preliminary selection, which were inoculated at the center of plates containing modified Czapek Dox agar supplemented with 0.01% asparagine and 0.009% phenol red. The L-asparaginase produced catalyzes the hydrolysis of asparagine, releasing ammonia and alkalinizing the medium, which causes the indicator to turn. The red halos produced were measured at 24, 48 and 72 h and the hydrolysis index was calculated by the ratio between the diameter of the hydrolysis zone and the diameter of the colony. *A. niveus*, *A. thermomutatus*, *F. lateritium* and *R. microsporus* var. *microsporus* had the best hydrolysis index, being, respectively, 2.8, 1.5, 1.82 and 1.33 for 72 h of incubation. The level of enzyme production is indicated semi-quantitatively by the hydrolysis index. Then, they were subjected to a qualitative test under submerged fermentation, in order to observe the production of L-asparaginase in a liquid medium. The modified Czapek Dox broth supplemented with 0.01% asparagine and 0.009% phenol red was used and the color change was observed. These fungi tested showed a red staining of the medium after 72 h, indicating the production of L-asparaginase. Considering these preliminary results, it is possible to infer that filamentous fungi are promising sources of L-asparaginase and may culminate in the production of an enzyme with fewer side effects, according to the pharmaceutical industry focus, emphasizing the importance of this study for the selection of a new enzymatic source.

Keywords: L-asparaginase, filamentous fungi, hydrolysis index.

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