TITLE: ENDOPHYTIC FUNGI OF *Vellozia Squamata* WITH POTENTIAL FOR PRODUCTION OF L-ASPARAGINASE

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ABSTRACT:
Acute lymphoblastic leukemia (ALL) is malignant neoplasm that affects the lymphoid lineage of the bone marrow. It is the most common malignancy in childhood, accounting for one-third of pediatric cancers. The treatment of ALL includes the use of L-asparaginase along other chemotherapeutics, to achieve better survival rates. In lymphocytic leukemias, there is a lack of production of asparaginase synthetase, and the amino acid is obtained from the plasma. L-asparaginase deprives these malignant cells of L-asparagine, by catalyzing the hydrolysis of L-asparagine in aspartic acid and ammonia, leading to cell death. Endophytic fungi appear to have a capacity to produce a wide range of enzymes, however, endophytes are poorly explored for biotechnological employment. In this study fifteen fungal isolates from *Vellozia squamata*, a common species in Cerrado areas were investigated for the production of L-asparaginase in a solid and liquid medium. The isolates cultured on PDA for 7 days were transferred to modified Czapek Dox Agar medium (with L-asparagine as nitrogen source) supplemented with phenol red, used as an indicator of the possible production of L-asparaginase (ammonia production). Plates without L-asparagine were used as control. The fungi were kept at 30 °C for 5 days and the presence of red halo in culture medium was evaluated, then the best L-asparaginase producers were chosen to grow in liquid medium (modified Czapek Dox). The isolates were incubated in a shaker for up to 7 days, at 120 rpm and 30 °C. The cultures were filtered and the periplasmic and extracellular enzymatic activity was evaluated using the hydroxylamine method. Of the fifteen isolates evaluated in a solid medium, four presented red halo across the board, contrasting with the controls: *Pestalotiopsis* (CCUB 555 and CCUB 564), Undetermined species (CCUB 562) and *Fusarium* (CCUB566). The periplasmic enzymatic activity of CCUB 566 was on average 0,492 U/g and the CCUB 555, CCUB 564 and CCUB 562 were 0,020 U/g, 0,031 U/g and 0,106 U/g respectively. The extracellular enzymatic activity had lower numbers, and none showed any activity greater than 0,007 U/mL. The production of L-asparaginase by *Pestalotiopsis* is still unknown. The isolates selected from solid-mediated screening had better periplasmic enzymatic activity compared to extracellular, but one new genus was discovered as producers of L-asparaginase.

Keywords: Acute lymphoblastic leukemia, L-asparaginase, Endophytic fungi, *Vellozia squamata*, Cerrado.

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