Molecular Modeling, Heterologous Expression and Gene Expression Analysis of NTPDASE-1 from Leishmania amazonensis.

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Leishmaniasis is a disease caused by parasites of the genus Leishmania, with approximately 12 million cases in the world. The forms of the disease in humans are cutaneous and visceral leishmaniasis. Leishmania amazonensis is the etiological agent of cutaneous leishmaniasis. NTPDases are enzymes located internally and externally in the cell and hydrolyze tri and/or diphosphate nucleotides, being essential for the acquisition of purines by trypanosomatids. This study aims to evaluate the role of NTPDase-1 through modelular modeling of this enzyme, the heterologous expression and gene expression. From the amino acid sequence obtained previously in this work, it was possible to predict the secondary structures using the PSIPRED server. Based on the tertiary protein structures obtained from the crystallized similar enzymes of Rattus novergicus, a comparative model for the LaNTPDase-1 was built. Expression analysis of LaNTPDase-1 through RT-qPCR and western blot were performed during a growth curve of promastigotes and between different evolutive forms (promastigotes, axenic and lesion amastigote). For the heterologous expression a set of primers was designed with and without N-terminal transmembranar helix. PCR products were cloned and the molecular characterizations of the recombinant protein are being conducted. The existence of a single transmembrane helix domain was predicted by the TMHMM software, at the N-terminal region, indicating that LaNTPDase-1 is a peripheral plasma membrane protein. There was a significant increase in gene expression from the 4th day and from the 5th day there was an increase in protein expression and between different evolutionary forms there was an increase in 5,2 and 7,3 to axenic amastigotes and amastigotes, in relation to the promastigote. These results suggest a compensatory cellular mechanism to enhance LaNTPDase-1 expression due to purine starvation in the medium at the long-period cultivation and an important role of this enzyme to the parasite-vertebrate host interaction.