Identification, Isolation and Cloning of the Nucleoside Hydrolase of *Leishmania amazonensis*

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Leishmaniasis is a disease caused by trypanosomatidae protozoa, endemic in tropical and subtropical regions of the world. The identification, knowledge and modes of interaction of essential enzymes of the parasite have become major targets for the design of new drugs. Nucleoside hydrolases (NHs) are metalloproteins responsible for the supply of purine and pyrimidine bases for the DNA and RNA synthesis, being essential for the development and survival of the parasites and it is not founded in mammals, making it a potential target for new drugs. Some NHs of protozoa had been characterized, like the NH of *L. donovani* (NHLd), however few of their corresponding genes have been identified. In order to identify this protein in the *L. amazonensis* genome we used our knowledge on the NHLd to isolate and clone the gene of NH of *L. amazonensis* (NHLa). We extracted the genomic DNA from the promastigote forms of *L. amazonensis* and the PCR was performed using oligonucleotides designed with the NHLd sequence. The isolated fragment with 1000 bp was sequenced with almost 100 % of identity with NHLd sequence. The cloning was performed in an express vector system, pET28b, with a histidine tag to facilitate the purification of the protein. The plasmid pET28b-NHLa was transformed into *Escherichia coli* BL21 and BL21 (DE3) cells for protein expression. These results enabled us to isolate a new target, until now unknown in *L. amazonensis* allowing further structural studies for the development of new drugs for chemotherapy of leishmaniasis.

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