MOLECULAR ANALYSIS OF LEISHMANIA APYRASES, CLONING INTO EXPRESSION VECTORS AND CHARACTERIZATION OF A PUTATIVE GDPase FROM L.major AS A GENUINE NTPDase

AMARAL,M.S.[1];COSTA.D.A.[1];AFONSO,L.C.C.[2]; MARQUES-DA-SILVA EA[2] VERJOVSKI-ALMEIDA, S.[3] and FIETTO,J.L.R.[4]

[1]NUPEB-UFOP-MG;[2]Laboratório de Imunoparasitologia-NUPEB-UFOP;[3]IQ-USP-SP;[4]DBB-UVF-MG

Leishmania major has two mapped apyrase genes in its genome (NTPDase and GDPase). Apyrase function, characterized as tri and di-nucleotide hydrolysis, were previously demonstrated in intact *L. amazonensis*, *L. braziliensis* and *L. major* cells. The very distinct ecto-nucleotidase capacity between *Leishmania* species suggests its involvement with virulence and control of host-immune responses. In order to evaluate if a molecular difference could explain these data we amplified, cloned and partially sequenced both genes from these 3 species. In addition, to perform further evaluations isolated full-apyrase genes were cloned into expression vectors (pET21b and pYES-CT). Analysis of partial genes and deduced proteins from *L amazonensis* and *L. braziliensis* showed 100% identity with *L. major* isoforms, suggesting that the different ecto-nucleotidase capacity could not be explained by only this molecular approach. The promastigotes expression profiles analysis showed high expression levels in *L. amazonensis* and suggested the presence of pos-translational modifications such as glycosilation. *In silico* analysis between *L. major* GDPase and NTPDase showed low identity (19.9%) and distinct putative sub-cellular localizations, soluble excreted and ecto-membrane respectively. The sub-cellular localization and glycosilation analysis will be the next steps in this investigation. Furthermore, the *L. major*`s GDPase heterologous expression, purification and characterization had been performed. The enzyme hydrolyzed some di- and tri-nucleotides, characterizing this enzyme as a genuine apyrase. Southern blot and complete characterization of these proteins could elucidate the real role of apyrases in *Leishmania* biology and virulence.

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