Production and Purification of Policlonal Antiserum Anti Recombinat GDPase from *Leishmania major*

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L. major has two mapped apyrase genes in its genome (putatives NTPDase and GDPase). Apyrase function, characterized as tri and di-nucleotide hydrolysis, was 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 (Maioli et al., 2004; Marques-da-Silva et al., 2008). The recombinant protein rGDPase was produced by heterologous expression in *E. coli*-pET21b system and the protein was purified using Ni-NTA-agarose affinity chromatography. To produce rabbit polyclonal antiserum we innoculated three sub-cutaneous injections containing 40µg of purified rGDPase. We used saponin (40µg) as adjuvant in the first and second injections. A pre-immune serum was collected as negative control sample before the first inoculation. To purify the antibodies, total protein extract from *E. coli*-BL21-pET21b without the GDPase was immobilized in a nitrocellulose membrane. The immune rabbit serum was placed overnight in contact with the membrane containing the bacterial extract to exclude contaminant antibodies. Then the serum was collected and used in western blotting assays. The western blotting tests using the purified protein and *E. coli*-pET21b cell extract showed that the serum is free from anti-*E. coli* antibodies. The use of this immune serum as a tool in *Leishmania* subcellular GDPase localizations and immunoprecipitation assays are prospects of this work.

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