

OLIGOPEPTIDASE B-LIKE FROM *L. AMAZONENSIS*

Guedes, H.L.M.^{1,2}, De Carvalho, R.S.N.¹, Rossi-Bergmann, B.² and
De Simone S.G.¹

¹FIOCRUZ, DBBM; ²UFRJ, IBCCF

Oligopeptidase B is an important virulence factor and therapeutic target in trypanosome infections. Genome project of *L. major* characterized a new oligopeptidase B, denominated oligopeptidase B-Like. In this work, a complete open reading frame of Oligopeptidase B-Like from *L. amazonensis* (PH8 strain) was amplified by PCR using primers designed for the Oligopeptidase B-Like gene from *L. major*. The 2175 bp fragment coded for a protein of 905 amino acids with a predicted molecular mass of 103918.9 Da and theoretical pI of 5.82. The encoded protein shares a 96 % identity with oligopeptidases of *L. major* and *L. infantum*, 94% with *L. braziliensis* and ~75% with Trypanosoma peptidases. By sequence alignment, we determined a catalytic triad (Ser 629, Asp 717 and His 758) and S1 subsite (Glu 674 and Glu 676) of oligopeptidase B-Like. The expression level of oligopeptidase B-like gene in m-RNA of promastigote (methacyclic and procyclic) and amastigote stages were determined by RT-PCR. The oligopeptidase B-like gene is expressed in all cycle stages of *L. amazonensis*. Gene copy number was determined by southern blot analysis in chromosome from promastigotes of *L. amazonensis*. Results suggest that oligopeptidase B-like gene is a single copy gene. Heterologous expression of recombinant oligopeptidase B-like in *E. coli* produced only insoluble protein in the inclusion bodies. Now, we are engaged in protein refolding procedures to further biochemical characterization.