MOLECULAR CLONING AND FUNCTIONAL CHARACTERIZATION OF AN ASTACIN-LIKE METALLOPROTEASE FROM *Loxosceles intermedia* (BROWN SPIDER) VENOM

Wille, A.C.M[1,2], Silva, D.T.[1], da Silveira, R.B.[1,3], Chaim, O.M.[1,3], Appel, M.H.[1], Senff-Ribeiro, A.[1], Gremski, W.[1,4], Nader, H.B.[3], Veiga, S.S.[1].

[1] Departamento de Biologia Celular, UFPR-PR.
[2] Departamento de Biologia Estrutural, Molecular and Genética, UEPG-PR.
[3] Departamento de Bioquímica, UNIFESP-SP.
[4] Instituto de Ciências Biológicas e da Saúde, PUC-PR.

Accidents caused by brown spiders are associated with skin necrotic lesions, gravitational spreading and systemic disturbances. Brown spider venom has a complex composition of toxins, of which metalloproteases have been described in this genus. Metalloproteases degrade extracellular matrix constituents then probably acting as spreading factor for the venom. Employing cDNA library of *L. intermedia* venom gland, we cloned and expressed a recombinant metalloprotease (LiRecMP - 900bp cDNA) that encodes for a signal peptide and a pro-protein. Nucleotide, deduced amino acid, phylogenetic analysis and computer modeling revealed a structural relationship with astacin family of metalloproteases. A recombinant molecule, expressed as an N-terminal His-tag was purified resulting in a protein of 30kDa. LiRecMP was refolded and used to produce a polyclonal antiserum that showed reactivity to LiRecMP and cross-reactivity to a native venom protein. Circular dichroism analysis evidenced correct folding of LiRecMP. Functionality was corroborated by its de-adhesive activity upon endothelial cells and hydrolysis upon fibronectin and fibrinogen (blocked by 1,10-phenanthroline) and upon gelatin. For the first time, an astacin-like toxin was described in a venom animal secretion and allowing further studies about venom and astacin family.