Molecular Cloning and Expression of Two Neurotoxins from the Venom of *Lasiodora* sp

Pimentel, F. G.; Mafra, T. B.; Pinto, I. A. A.; Dourado, V. A.; Brandão, R. L. and Castro, I. M
Laboratório de Biologia Celular e Molecular – NUPEB / UFOP - Ouro Preto – MG - CEP 35400.000

The venom of spiders, scorpions and cone snails are particularly interesting from a pharmaceutical and agrochemical perspective because of the diversity of gene-encoded peptide toxins that are expressed in the venom glands of these animals. The targets of these peptides are ion channels of excitable and non-excitable cells. The venoms of *Lasiodora* spiders are a source of pharmacologically interesting toxins active against Na^+ and Ca^{2+} channels but until now these venoms have not been studied systemically. In a preliminary study, the cDNA library constructed from venoms glands of *Lasiodora* sp was screened by the use of ELISA with the aim to identify immunogenic proteins. Five cDNAs encoding the precursors for the toxins LTx1-LTx5 were characterized by sequencing. The two mature toxins LTx4 and LTx5 have completely different primary structures from the toxins LT1-LTx3. Recent study revealed that recombinant LTx2 acts on calcium channels, blocking L-Type calcium channels. The aim of the present work was to characterize the toxins LTx4 and LTx5. LTx5 expression was induced in *Saccharomyces cerevisiae* W303, after the transformation of yeast cell, by addition of 2% (p/v) galactose to cell culture; LTx4 was induced in C41 (DE3) cells by IPTG addition. The toxins were immunodetected by anti-His as the primary antibody. The purification of recombinants peptides by Microcon Centrifugal Filters and Ni-NTA resin are in course. The expression level of LTx5 was very low. Dot blot assay has shown that recombinant LTx4 is mostly expressed as inclusion bodies.

Supported by CAPES, CNPq ,FAPEMIG, UFOP