Molecular characterization, immunological analysis and heterologous expression of phospholipase A1 from wasp venom of Social *Polybia paulista* (Hymenoptera, Vespidae).

Campos-Pereira, F.D.\(^1\); Jacomini, D.L.J\(^1\); Giratto, D.T.\(^1\); Silva Neto, A.J.\(^2\); Zollner, R.L.\(^3\); Brochetto-Braga, M.R.\(^1\)

\(^1\) Dept of Biology, UNESP, Rio Claro, SP, Brazil.
\(^2\) Faculdades Integradas Claretianas, Rio Claro SP, Brazil.
\(^3\) Dept of Clinical Immunology and Allergy, Faculty of Medical Sciences, UNICAMP, Campinas, SP, Brazil

*Polybia paulista* (Hymenoptera, Vespidae) is very common wasp in southeastern Brazil. Its venom is composed by amines, peptides and allergenic proteins and enzymes, being the phospholipases (PLAs) the most abundant and important allergens. The complete PLA1 cDNA sequence (985 bp) from *P. paulista* venom was cloned and sequenced. The deduced protein belongs to the esterases lipases Super Family, posses 302 amino acids, a theoretical pI of 9.1, MW of 33 kDa and its active site was identified between residues 131 to 140. The Rec-PLA1 with a 6x histidine tail at the N-terminal was expressed in *Escherichia coli* BL21-DE3 using the pET-28a vector and recovered in inclusion bodies. The purification was performed on agarose-Ni\(^{2+}\) affinity resin with 8M urea and the protein was eluted with a decreasing step-wise pH buffer (6.3 to 4.5). Besides, polyclonal antibodies were produced in mice, against an electrophoretic fraction of natural PLA1 from the crude venom and the Western blotting analysis revealed that the corresponding natural PLA1 protein band and its isoforms were very specifically recognized as well as the Rec-PLA1, indicating that some epitopes are still accessible in the recombinant protein. In addition, as already described to other insect venoms, these antibodies also displayed immunological cross-reaction with crude venom of other social wasps tested here. These novel results besides open perspectives for explanations of the biochemical and immunological action mechanisms of the cross-reactivity of the venom allergens are very important by its potential use in allergies diagnostics and allergen-specific immunotherapy using our neotropical insects.


Supported by: FAPESP, CAPES and FUNDUNESP