# cDNA CLONING, EXPRESSION AND TRANSCRIPTION ANALYSIS OF SERINE PROTEASES INHIBITORS of *Triatoma infestans* EGGS

deMarco, R.[1], Campos, I.T.N.[2] and Tanaka, A.S.[1]

[1]Departamento de Bioquímica, UNIFESP, São Paulo, SP, Brazil.
[2]Departamento de Bioquímica e Biologia Molecular, UFG, Goiânia, GO, Brazil. E-mail: demarco36@gmail.com

In the last decade, a new serine protease inhibitor family called Pacifastin has being described. It is characterized by presenting low molecular weight and the following motif **Cys**-Xaa9-12-**Cys**-Asn-Xaa-**Cys**-Xaa-**Cys**-Xaa2-3-Gly-Xaa3-6-**Cys**-Thr-Xaa3-**Cys**. Recently, we purified two Pacifastin-like inhibitors found in the *Triatoma infestans* eggs. They inhibited neutrophil elastase and chymotrypsin and presented the same N-terminal sequence. We described for the first time a Pacifastin inhibitor in a blood-sucking bug species. The cDNA fragment coding for two pacifastin inhibitors was amplified from a *T. infestans* fat body cDNA preparation. Its translated amino acid sequence codified for two Pacifastin inhibitors *in tandem*. These domains were separately cloned in the vector pPIC9 for expression in *P. pastoris* yeast. The second pacifastin domain, TIPI2, did not express, but the first pacifastin domain, TIPI1, was expressed in a high level (3,8 mg/l). TIPI1 purified on a chymotrypsin-Sepharose presented inhibitory activity for chymotrypsin (Ki = 4.96 nM) and neutrophil elastase (Ki = 0.41 nM), similar to the native inhibitor. In this work, we also showed the transcription profile of TIPI inhibitors after blood feeding by Real-Time PCR, which presented a high transcription level at 36 h after blood meal. Our perspective is to localize the native protein in different tissues of *T. infestans*. *Financial supported by: FAPESP and CNPq*.