## EXPRESSION, PURIFICATION AND CHARACTERIZATION OF A KAZAL-TYPE SERINE PROTEASE INHIBITOR FROM AEDES AEGYPTI SALIVARY GLAND

Watanabe, R.M.O.<sup>1</sup>, deMarco, R.<sup>1</sup>, Gaio, A.O.<sup>2</sup>, Lemos, F.J.A.<sup>2</sup>, Tanaka A.S.<sup>1</sup>

<sup>1</sup>Departamento de Bioquímica - UNIFESP - SP, Brazil; <sup>2</sup>Laboratório de Biotecnologia - UENF - RJ, Brazil. E-mail:renatamidori19@gmail.com

Aedes aegypti is an important vector of diseases such as yellow fever and dengue fever. Serine protease inhibitors play crucial roles in controlling host physiological processes such as: blood coagulation, complement activation, fibrinolysis, immune response, by inhibiting target enzymes involved in these processes. In order to better understand about vector's hematophagy adaptation and reproduction, our aims were expression, purification and characterization of a novel Kazal-type serine protease inhibitor from Ae.aegypti salivary glands. Our strategy was construction of oligonucleotides based on the nucleotide sequence no.DQ440176 (GenBank). Using the oligonucleotides and a PCR product kindly provided by Dr. J.M.Ribeiro (NIH -Rockville, USA), a new PCR fragment was generated, which was cloned into pET-26b(+) vector. Recombinant serine protease inhibitor (AaTi) was expressed in E.coli BL21-SI, by induction with NaCl. After expression, the cells harvested by centrifugation, were lysed using a French press. The cell lysed was centrifuged and the supernatant used in the purification by affinity chromatography. The purified AaTI inhibited trypsin with Ki of 1.2 nM, but it did not inhibited thrombin, fXa, plasmin, tissue plasminogen activator, urokinase, fXIIa and plasma kallikrein. The perspective of this work is to improve the AaTI expression level to obtain enough material for its biochemical and physiological studies. Supported by: FAPESP/CNPg.