KUNITZ-BPTI LIKE PROTEIN FROM cDNA LIBRARY OF *Boophilus microplus* HEMOCYTES*:* CLONING, EXPRESSION AND PARTIAL BIOCHEMICAL CHARACTERIZATION

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Several hematophagous are known as disease vectors; they possess effective coagulation and encapsulation processes, which are adapted to the properties of an open circulatory system and also for pathogens eliminations. Soluble factors and hemocytes different types participate in these processes. To identify molecular components of the tick immune system, a *B.microplus* hemocytes cDNA library was constructed using ticks challenged with *M.anisopliae* fungi and Smart-system. We sequenced 570 isolated clones, among them 35% were implicated in tick immune system as: lysozymes and antimicrobial peptides. Fifteen ESTs (3%) corresponding to a Kunitz-BPTI-like inhibitor containing a predicted signal peptide (22 amino acids) in a sequence of 58 aminoacids with theoretical pI and Mr of 9.2 and 6.5kDa, respectively. The Kunitz-BPTI-like was cloned into pPIC9 vector and the rBmCI expressed in *P.pastoris*. The recombinant BmCI (*B.microplus* chymotrypsin inhibitor) was purified by size-exclusion chromatographies (Superdex75). Purified BmCI strongly inhibited chymotrypsin (Ki6.0pM), and also affected neutrophil-elastase (Ki7.0nM) but did not Proteases (Pr1), (Pr2), trypsin and subtilisin A. By semi-quantitative RT-PCR an increasing BmCI transcription after immune induction was detected. The BmCI transcription (174bp) was confirmed in ovary, salivary glands, unchallenged hemocytes, but not in the fat body. Purified BmCI did not present any antimicrobial activity on *E.coli* and *S.cerevisiae*. The perspectives are to study a possible synergism with hemolymph molecules and BmCI, and the inhibitor role in the tick immune response.

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