

## **ANALYSIS OF A SERINE PROTEASE GENE EXPRESSED IN MIDGUT OF AFRICAN TRYPANOSOME VECTOR, *GLOSSINA FUSCIPES FUSCIPES***

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Serine proteases are major midgut enzymes that have been implicated in the process of pathogen establishment in several vector insects. Using highly degenerate trypsin-specific PCR primers, a 458bp cDNA probe was generated that showed similarity to a serine protease fragment of the blowfly, *Lucilia cuprina* with an expectation of  $2 \times 10^{-62}$ , 73% identities and 84% positives. We have now screened a *Glossina fuscipes fuscipes* cDNA midgut expression library using this probe and identified a full-length serine-protease gene.

The cDNA encodes a putative mature polypeptide with 269 amino acids with a predicted molecular weight of  $\sim 29\ 245$  Da. The typical features of serine protease trypsin family of proteins found in the sequence include the His/ Asp/ Ser active site triad with the conserved residues surrounding it, and three pairs of cysteine residues for disulfide bridges. In addition, the sequence contained several potential phosphorylation sites. Expression of the gene in a bacterial expression system yielded a protein with molecular weight of  $\sim 26\ 807 \pm 2748$  da. The recombinant protein had trypsin activity of  $4.390 \text{ units} \times 10^{-4}$  and exhibited remarkable lysis activity against bloodstream trypanosomes and rabbit RBCs *in vitro*. These results support our earlier hypothesis that this molecule might be involved in the establishment of trypanosome infections in tsetse.

Keywords: Tsetse, *Glossina*, serine-protease, trypsin, trypanosomes