Production of a New Antistasin-family Molecule from *Haementeria depressa* Leeches

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Some new molecules able to act in hemostatic system have been discovered through of saliva of bloodsucking animals. Studies from *Haementeria depressa* leeches salivary complexes have been determined a profile of transcripts and proteins produced by it. The H05D10_pGEM11Zf is a transcript similar to therostasin, an antistasin-family member, FXa inhibitor. This clone was previously expressed in prokaryotic system unsuccessfully. The aims of this work were obtaining the recombinant molecule by *P.pastoris* expression, and to characterize the isolated molecule by activity screening.

H05D10 was subcloned in pGEM-Teasy to finally clone in pPIC9K between EcoRI and NotI cloning sites. *P.pastoris* (GS115) was transformed with the recombinant vector previously linearized by SacI and the expression was done in BMG and BMGY mediums on 28º - 30ºC, after that, some methods of purification including dialysis, heparin-sepharose and gel filtration chromatographies were realized; the search of inhibitory activity was assayed on FXa. The cloning was successfully done and the expression was standardized as well. The partial purification of the recombinant protein was obtained by heparin-sepharose chromatography and it was eluted in NaCl 550mM, this step showed two bands on SDS-PAGE, the major band had about 13kDa as expected. The partially purified molecule (~ 3µg) was able to inhibit 65% of the FXa activity on chromogenic substrate (S2765). We intend to improve the purification, to characterize the probably specificity of protein on inhibit FXa. This new antistasin-family member is a potential anticoagulant to be explored in further investigations.

Keywords: FXa inhibitor, antistasin-family, *P.pastoris* expression

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