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Bachelor thesis evaluation: Markéta Absolonová
Theme: IrAM4: Partial characterization of molecule similar to α_2-macroglobulin from a tick *Ixodes ricinus*

The bachelor thesis of Markéta Absolonová describes IrAM4, a member of thiolester protein family from the hard tick *Ixodes ricinus* and is written on 22 pages. The thesis consists of 4 chapters (introduction, materials and methods, results and discussion, conclusion) and has a good quality of English with minimum of typing and spelling errors.
The first chapter, named Introduction has three pages and the author very briefly characterizes ticks, their life cycle and members of α2-macroglobulin protein family. In mine opinion, this part is too short and could be more extensive.
I have two reminders to this chapter: Firstly, the hard plate of *I. ricinus* is not named sputum but scutum (page one).
Secondly on the same page, the author has written that one of the features of α_2-macroglobulin family members is the presence of reactive thiolester bond. This is not entirelly true, because it is known, that some proteins (for example *Drosophila melanogaster* TEP6, *I. ricinus* IrAM 2 and 8, vertebrates complement factor C5, etc.) lack this bond.
Chapter two: Materials and methods. The author describes many molecular and biochemical approaches, such as RNA isolation, cDNA synthesis, expression of recombinant protein and others. All methods are described very shortly and according my opinion could be defined in more details. I am not sure, if student of bachelor degree will be able to follow and accomplish all methods according authors description.
Chapter three: Results and discussion: Results and discussion are connected into one chapter and are written on eleven pages and include ten images of good quality. It is obvious from this chapter, that the author obtained a nice piece of work. I have few questions and recommendations.
First, page twelve, the author performed RT-PCR of IrAM4 in gut, salivary glands and ovaries and did not incorporate hemocytes due to low amount of cells for isolation of RNA by Tri Reagent solution. This could be easily solved by use of commercial kits for RNA isolation of small volumes (eg. RNeasy® Micro kit, Qiagen).
Second, page thirteen, I am missing any information, which says something about expected (predicted) size of IrAM4 amplicon, that was consequently used for clonig and production of recombinant protein.
Third, page twenty, gained antibody does not appear to be very specific as we can see in figure 11(Western blot), where several bands of various sizes have been detected. As author writes one of the possibilities to verify the real size (band) of IrAM4 protein is to use RNA interference. Another alternative is to purify specific antibody using IrAM4 recombinant protein coupled to the CNBr-activated sepharose and re-run the WB.
The last question, did the author perform all experiments by herself?

Through the above comments and questions, it is obvious, that Markéta managed many methods and got relatively large amount of results. According my opinion, the Bachelor thesis of Markéta Absolonová meets all requirements of Faculty of Science at University of South Bohemia and therefore I recommend it for the defense.

In České Budějovice 13.6.2011

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