Přírodovědecká fakulta
Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

OPPONENT'S REVIEW ON DIPLOMA* THESIS

Name of the student: **Bc., BSc Hana Pechová**

Thesis title: Analysis of trypsin inhibitor-like cysteine-rich domain-containing peptides (TIL-domain inhibitors) from the tick *Ixodes ricinus*

Supervisor: **Mgr. Jan Kotál Ph.D.**

Referee: RNDr. Zdeněk Franta Ph.D.

Referee`s affiliation: **UCH**

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced length of the thesis parts** (recommended length of the theoretical part is max. 1/3 of the total length), **logical structure of the thesis**	0-3	3
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	2,5
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
Graphic layout of the text and of the figures/tables	0-3	2
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	2,5
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	1
Formal requirements – points in total		17
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	3
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	2,5
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	2,5
Logic in the course of the experimental work	0-3	3

* Choose one

[1] Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Completeness of the description of the used techniques	0-3	2
Experimental difficulty of the thesis, independence in experimental work	0-3	2
Quality of experimental data presentation	0-3	2
The use of up-to-date techniques	0-3	2
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	2
Practical requirements – points in total		21

POINTS IN TOTAL (MAX/AWARDED)	48	(38)[2]

Comments of the reviewer on the student and the thesis:

Presented thesis of Hana Pechová deals with the interesting topic of trypsin inhibitor-like cysteine-rich domain-containing peptide from *I. ricinus*. The thesis is written on 84 pages and is divided into 8 chapters. The thesis is in general written in good English and contains only few typos and spelling errors. With the exception of Material and methods chapter, all the others are quite well organized. The chapter M and M seems to me written in a bit hurry. It contains more typos than the other parts and several important information about the methods used by the author are incomplete and make it hard to repeat the experiments (eg. Subchapter 3.2.4 Cloning of TIL-1, subchapter 3.2.8 Protein purification or subchapter 3.2.6 Western blotting contain only few information about the used procedures and require further improvement).

As author mentions tick are long feeding ectoparasites, which exploits broad portfolio of biologically active molecules with pharmacological potential and studies dealing with identification and characterization of these molecules are of broad interest. In this thesis author explores the available transcriptomic databases of *I. ricinus* and search for the TIL-domain inhibitors. As a result the author identifies 78 sequences which she divided among 17 clusters (families).
Author chose one candidate gene (TIL-1), and used swiss model and LOMETS to predict its crystal structure and compare it with three available structure of AMCI - *Apis mellifera* chymotrypsin inhibitor, Ascaris TIL inhibitor complex ad *B. bombina* BSTI trypsin inhibitor. In the next part of the diploma thesis author cloned the TIL-1 gene into *E. coli* expression vector, produced and purified the recombinant protein and tested its inhibitory potential using various clotting assays. Finally, the author analyzed the tissue expression profiling of the TIL-1 gene using quantitative PCR. From the presented thesis it is obvious that author spend quite some time in the lab and handled various techniques, however the acquired results require further investigation and confirmation.

[2] Enter the number of points awarded.

Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future:

1 – In the figure 2 you show that serpins could form polymers. Is there anything know about the role of these polymers?

2 – In introduction page 11 you state that Kazal-domain inhibitors can defend parasite from microbial or host digestive proteases. Could you please explain more precisely how would this work?

3 – You did choose *E. coli* expression system for the production of TIL-1 peptide, but this peptide contains 5 dissulfide bridges and its production in *E. coli* can be quite complicated. Have you consider any other system or peptide synthesis, which could be relatively cheap for the short peptide.

4 – There is 4 nucleotides difference between the gene specific primers sequence used for Nebuilder and Champion pET cloning. But this sequence, in my eyes, shall be the same. What is this difference for?

5 – On page 23 you state that the cDNA from salivary glands was used as a template for PCR amplification. Why did you start with this cDNA if the highest gene expression is in the tick gut? Cannot this be the reason for complication with cloning and quite low PCR amplicon as seen in figure 15?

6 – how specific are qPCR primers towards the other TIL families? Can you make the nucleotide alignment and show where the primers sit? I am also missing the information about the melting curve analysis here.

7 – from the AA sequences it is obvious that TIL-1 is not very similar to any of inhibitors, which were used to model TIL-1 structure. As I am not an expert in in silico protein modeling, just would like to ask you, if it is possible to get a good structure prediction if my protein is not very conservative and differs from the template.

8 – Pilot expression is depicted in figure 17. It is quite hard to read the results. It is not clear, whether author loaded soluble or insoluble fraction, I am also missing the non induced control, which will confirm the overproduction of target protein. Moreover author tested various time, but for the large scale production she used different time. Was there any reason for it?

9 – From my own experience, the cultivation temperature of 30°C and 37°C would not change the protein production so much. E. coli cells grown kinetics is very similar at these temperatures. To decrease the growth time (which is often associated with better protein folding) I would recommend to grow cells at much lower temperature (≤ 20°C).

10 – it is very hard to confirm the presence of TIL-1 on the WB picture in figure 18. The blot is very blury and almost unreadable, also from your SDS-PAGE gel I do not see much. Could you show better pictures for the presentation? Moreover from all the SDS-PAGE gels presented in the thesis I am not very confinced that you did produce the protein of interest. The size of the band from the gel does not really correspond to the predicted size (15kDa vs 24kDa). Have you tried to verify the presence of TIL-1 by any other method?

11 – I would recommend to add chromatograms for individual purifications. This would help the reader to get the information about the purification process.

12 – in figure 22 you show the results of your 2nd affinity purification and elution of pET SUMO upon cleavage. Have you try to test the presence of SUMO tag via the WB. It is a bit weird that 6xhis SUMO would elute with 20mM imidazole.

13 – The authors discuss the efficacy of SUMO-tag on page 60. I would here just stress the point that the SUMO tag is primarily added as a folding chaperone and not due to its 6-his tag on N- terminus (as I did understand it from the discussion).

Completeness of the description of the used techniques	0-3	2
Experimental difficulty of the thesis, independence in experimental work	0-3	2
Quality of experimental data presentation	0-3	2
The use of up-to-date techniques	0-3	2
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	2
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[2] Enter the number of points awarded.

Conclusion: Despite all my comments and question I am convinced that the presented thesis fulfills all the criteria of Faculty of Science at University of South Bohemia and I have no hesitation in recommending the thesis of Bc. Hana Pechová for Mgr. defense

In conclusion, I

r e c o m m e n d *

the thesis for the defense and I suggest the grade 2 .[3]

In **České Budějovice** date **20.01.2021**

[3] You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).