Přírodovědecká fakulta
Faculty of Science

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

OPPONENT'S REVIEW ON BACHELOR/~~DIPLOMA~~* THESIS

Name of the student: Agop Kabrelian

Thesis title: Reverse pharmacology: Searching for novel drugs in ticks and testing their effects in host immune cells.

Supervisor: RNDr. Jindřich Chmelař, Ph.D.

Referee: RNDr. Ján Štěrba, Ph.D.

Referee`s affiliation: Institute of Chemistry, Faculty of Science, University of South Bohemia

	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
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	1
Graphic layout of the text and of the figures/tables	0-3	2
Quality of the annotation	0-3	1
Language and stylistics, complying with the valid terminology	0-3	1.5
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	2
Formal requirements – points in total		12.5
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	1
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	1
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	1
Logic in the course of the experimental work	0-3	1

* Choose one

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

Completeness of the description of the used techniques	0-3	1
Experimental difficulty of the thesis, independence in experimental work	0-3	1.5
Quality of experimental data presentation	0-3	1
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	1
Practical requirements – points in total		11.5
POINTS IN TOTAL (MAX/AWARDED)	48	24

Comments of the reviewer on the student and the thesis:

Overall:

Many missing words, incorrect words, incorrectly used commas. You should decide if you are using the Oxford comma or not and do it in the whole text the same way.

Be aware of the different use of a hyphen (-) and a dash (–), together with the use of spaces around these two different characters. You should use a non-breaking hyphen and non-breaking space when needed (e.g. Da-p36 on page 10, 1 ml on page 17).

The Latin name of the genus mentioned for the first time is written in full; in all the next occasions (except when at the beginning of the sentence) it is abbreviated into one-, two- or max-three-letter abbreviation. Latin names of organisms are always written in italics.

You should cite a source for every graphics taken from some other source in the figure caption, e.g. Fig. 5. Figure caption must be on the same page as the figure (Fig. 2 – pages 2 and 3).

The main thesis parts (Introduction, Aims, Material and Methods, ...) usually start on a separate page. The different levels of section headings should be written in different font sizes, the same level in the same sizes ... (see, e.g. section headings 3.2.3 and 3.2.4).

You should learn the writing of spaces between the number and the unit (for example the difference in the meaning of 72°C and 72 °C).

You should write correctly the names of organisms, especially of those, which you are working with. E.g. *I. scapularies*, page 15. Also, at least the names of the proteins you are working with should be written correctly (Da-p35 in Table 3 caption).

Annotation: "Ixodes ricinus salivary", discrepancy between the English and German annotation ("salivary" vs salivary glands and midgut).

Introduction:

Every figure and table must be cited in the text (e.g. Fig. 1, Fig. 5 are not mentioned anywhere in the text). If you are not mentioning in the text, then it means it is not important for the text and thus why to use it in the thesis?

Figure 4 – Are the 454, Solexa, and Sanger still the main methods of choice for massive sequencing today?

Aims:

To gain experience with many methods should not be an aim of a bachelor thesis.

The aims should be consistent – you are writing about one protein, then several, then one again.

Materials and methods:

Do not forget to write correctly chemical formulas; you are a chemist!!!! (e.g. Na3N, H2O)

You should include the source of all the purchased chemicals, solutions – including the RPMI 1640, and also the composition of the used solution – such as the Trypan Blue solution, complete info on Tyrode's buffer, HBSS (this abbreviation is not explained).

PCR – from your description, I got an impression, which you do not really understand the method (confirmed even more in Discussion). You should pay much more attention in correct writing and explanation of methods and procedures (this is not the only case).

You should use some understandable names of proteins in phylogenetic trees, or at least explain what is what in the caption. Otherwise, it is worthless to include such a phylogenetic tree in your work.

Results: many descriptive parts of the Results section should be part of the Methods.

References:

Journal titles are always written in capital letters and are written either in full or in abbreviations – use only one of these styles.

Article titles are either written in capital letters or not – you cannot mix these two styles.

Latin names of organisms are even in references written in italics.

The same style of citation must be used in the whole thesis/work.

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

As a thesis should be written in such a way for any other reader to be able to repeat the experiments, I have a bit more questions, which should give the missing information. Still, there is much more question, but there is no time during the defence to answer all of them.

1) Which of the bioinformatic procedures were performed by you and which by somebody else? At least some part was performed previously, as you write in the Conclusion.
2) Which parameters for gap opening and gap extension penalties were tested (page 15)? And you write about removing of signal sequences before the analyses. Which analyses?
3) Page 16 – cDNA production – why are you writing about cDNA production if the cDNA was provided by Jan and Michalis??? Why did you not produce the cDNA by yourself, if you wanted to gain experience with many methods (written in Aims)?
4) Page 16 – PCR – you are not writing about the volumes of the reagents, primers, water added into the PCR reaction (both Taq and Phusion). Could you specify?
5) Page 25 – you are writing about cloning, but there is no cloning described in the methods and later you write, that you did not do any cloning. So, what is correct?
6) Page 17 – 3.2.4 – what kind of 96-well plates was used for coating with fibronectin? At the end of the procedure, you are writing about fluorescence intensity between washed and unwashed wells – anyway, you are not mentioning wells washing. What do you mean by that?
   Discussion vs Methods – so, how many and which proteins did you use to coat the wells prior to leukocyte adhesion?
7) Why was only IRS-1b tested in a high salt buffer?
8) Why various concentrations of the tested proteins were used in the different

experiments? (1 μM in 3.2.4, 3 μM or 6 μM in 3.2.5, 2 μM in 3.2.6.2)

9) Could you again explain the platelet aggregation experiment 3.2.6.2? If you stained one part of the platelets with one dye, the other with another, then mixed these and just after that you added the tested proteins, what was the result of the experiment? Is the described procedure correct?

10) The list of the identified proteins similar to Da-p36 (4.1) is missing – please, show this list (the identified 10+21+42=73 proteins). Why were they divided into three groups/families: Da-p36, Da-p36 like and Cytotoxin Da-p36? In *D. andersonii*, as I understand, only one (an isoform of) Da-p36 is present and produced, right? Then, the ten identified members of the Da-p36 in *I. ricinus* are ten isoforms present in the genome or 10 variants of the same gene from various ticks?
Later, you are writing only about one member of the Da-p36 family in *I. ricinus* – which one of all those mentioned previously (10+21+42 proteins) and how was it selected?

11) You are writing on page 21 – "Different isoelectric points insure efficient purification of proteins by isoelectric focusing" on the different pI of the Da-p36 and one of the putative Da-p36 from *I. ricinus*. Why is it important? You expect to mix proteins from two different ticks and then to purify one or the other or why do you mention it?

12) Fig. 8, 9 – which proteins are shown and how were they chosen? You write about the Da-p36 family, but that should have only ten members in *I. ricinus* (page 21); the same for phylogenetic tree.
What is the difference between the groups 1 and 2 from Fig. 9, which proteins belong there, etc. ...?
Based on your results – the final conclusion is, that I. ricinus does not contain Da-p36, correct?

13) Page 34 – Discussion – You write "Also, from table 2 we see that *I. ricinus* Da-p36 proteins do not belong to the same family with original one". Tab. 2 is a list of proteins used in immunoassays. Could you explain?

14) Show us please Fig. 10 with the marker bands described IN THE FIGURE (sizes of bands) and also add a description for the samples analysed – it is not possible to understand from your figure and caption. Which protein was amplified? Did you use 1 bp marker (caption) or 100 bp marker (figure)? If the Da-p36 should be expressed mostly in salivary glands, how do you explain, that you got positive PCR result only from the ovarian cDNA? Did you use salivary cDNA or salivary gland cDNA?

15) Why did you not try to amplify some of the other putative Da-p36 genes, try different PCR mixtures, different conditions etc. and instead proceed to some other methods – for which you did not have your own protein? You write that the work aimed to walk through all steps ..., but obviously you skipped one of the most important steps (amplification) and because of that another two steps (cloning, recombinant protein production). Did you at least produce the recombinant proteins used in your thesis by yourself from the plasmids?

16) Page 19 – 3.2.5 – for FACS, you stained the same cells with PE and PI? How did you distinguish the signal? You are writing about the lasers for excitation – did you use both? And what about the filters for emission (channels)? Which channel(s) did you use?

17) Fig. 13 – are you sure the black shows all the events? And are the red really parent cells and green singlets? Could you explain the figure? In methods, you wrote about PE and PI dyes used for staining of the cells, here FITC and PE-Texas red channels are shown – what is correct? In Fig. 14 you are then using the APC channel yet

increasing the confusion. Consequently, I do not believe your results in Fig. 15 at all.

18) Page 32 – in three consecutive sentences you write: "We see no difference in platelet aggregation ...", "showed slight stimulation of aggregation", "A slight inhibition". So, was there a difference or not? Why was the experiment done only once? Again, it is just showing you tried to do a lot of methods but without getting real experience and results – that is worthless in science!

19) As you write, you had just a short time for the project (that is disputable). Then, why did you start a totally new assay in the lab (as you write for platelet aggregation experiment) instead of working on some more basic methods, which did not work in your hands???

Overall, it seems that you tried to gain experience in too many methods (as stated in the Aims), but you did not learn well any of them, which is a bad approach. Much more effort should be put in your future work into an in-depth understanding of just a few methods and the ability to write a good summary of your work. You also state in the Discussion "I performed several experiments and analyses ..." – it is not true, you tried to perform several experiments, but mostly they did not work well, or you did not understand what you did (as you show in the Methods and Results). And I am not sure, how much of the bioinformatics part was done by yourself.

Conclusion:

In conclusion, even despite many reservations I

r e c o m m e n d / ~~d o n o t r e c o m m e n d~~*

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

In **České Budějovice** date **7. 9. 2018**

.....

signature

[2] 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).