Přírodovědecká fakulta
Faculty of Science

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

OPPONENT'S REVIEW ON BACHELOR THESIS

Name of the student: Jakob Schmelzer

Thesis title: Expression and purification of tick-borne encephalitis virus capsid protein
Supervisor: RNDr. Zdenek Franta, PhD.
Co-Supervisor: Paulina Duhita Anindita, DVM, PhD.

Referee: Mgr. Hana Tykalová, PhD.
Referee`s affiliation: **University of South Bohemia in Ceske Budejovice, Faculty of Science AND Biology Centre, Czech Academy of Sciences, Institute of Parasitology**

	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	2
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	1.5
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	1
Graphic layout of the text and of the figures/tables	0-3	1
Quality of the annotation	0-3	2.5
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 labelling, indication of the units)	0-3	1
Formal requirements – points in total	**21**	11.5
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfilment of the aims	0-3	0
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	1.5
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	3
Completeness of the description of the used techniques	0-3	2

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

Experimental difficulty of the thesis, independence in experimental work	0-3	3
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	2
Practical requirements – points in total	**27**	16.5
POINTS IN TOTAL (MAX/AWARDED)	**48**	28

Comments of the reviewer on the student and the thesis:

The Bachelor´s thesis by Jacob Schmelzer entitled "Expression and purification of Tick-borne encephalitis virus Capsid protein" deals with cloning, expression, and purification of the structural component of the TBEV nucleocapsid. The thesis is written in decent English coping with the proper scientific terminology. I would also highlight the wide range of methods that the student has mastered and the multitude of optimization conditions tested during the experimental work. I appreciate that he was able to achieve at least partial success in purifying such a hard-to-express protein, which TBEV C is.

As a whole, the Bachelor´s thesis meets most of all the formal requirements, with the exception of the missing aims of the thesis. However, since the beginning, it is obvious that more attention should have been devoted to proofreading. Already on the title page, there are capitalization errors in the title and the page is numbered. A high number of negligence mistakes are present all over the thesis as well as problems of coherence appear, and the format of tables is not unified. It is also obvious, that initially, the goal was to express and purify (also) the anchored version of C protein and that the idea to include this to the thesis was abandoned at some point, but the remains of that are present in several places (e.g. section 2.1.1., 2.1.8, figure 7, table 3-2). For example, Mr Schmelzer states, that the C-anch was cloned previously (p. 18), but in figure 7 shows the amplification result where he optimized amplification conditions for cloning. However, primer for doing so is missing in the method section (table 2-1, page 8). I suggest either leaving out the part of C-anch completely or including it with proper reasoning already in the method section for example. The extent of the introductory section is appropriate, even though I can imagine a more detailed review of the C protein functions in the TBEV as well as in flaviviruses in general (section 1.5). The description of methods is most detailed and with fewer mistakes than the rest of the thesis and the average summary of results follows. The candidate should improve the quality of the discussion. Although the student cites relevant sources and extracts appropriate data from the literature, critical comparison with the research is missing as well as there is a lack of suggestions for the improvement of the protocol in the future so that better results can be gained.

I will not address every single issue I found in the thesis, I will just give here a brief overview of the types of mistakes I have encountered during the review.

Major issues:

- The absence of the aims of the thesis does not allow me to evaluate whether they were met.
- Figure 6 is overtaken from Oliviera et al. 2017, but this is not dedicated in the figure legend.
- Missing data in table 2-7
- Cited literature could be more up-to-date. About 40% (18 publications) of the cited literature is older than 10 years.

Minor issues:

- Wrong capital letters usage
  -please follow the official nomenclature to use the capital letters in the name of viruses correctly: T/tick-borne encephalitis virus (TBEV), Dengue virus (DENV), Zika virus (ZIKV), Yellow fever virus or West Nile virus (WNV)
  - Random or wrong capital letters usage in titles, names of procedures, figure legend headers or in table contents (e.g. “Gel Electrophoresis”, “Nitrogen” p. 14)
- Inconsistent nomenclature – the same step of PCR cycling is called either “Final Elongation or Final extension”.
- Inconsistent formatting of tables (e.g. tables 2-11, 2-12 compared to 2-6)
- Incomplete descriptions of figures, tables or legends (e.g. figures 17, 19)
- Wrong or missing dedication to figures in text (e.g. page 19 should state 2.1.6 not 3.1.6)
- Missing composition of TAE buffer (page 9)
- Missing reference sequence number for the target sequence amplified, sequence is provided in the appendix section but without any reference in the thesis
- Primer R2 (2-1) does not match the target sequence (TTA) stated in the appendix
- rpm values without the rotor radius are not a correct reference of centrifugation speed, use centrifugation speed expressed in g instead.
- Figures are numbered continuously throughout the thesis (1-19) but tables are numbered by the section (first table is thus 2-1).
- In the reference section many inconsistencies in the format of references (e.g. missing italics in journal names or Latin names (E.coli), absent capitalization of journal names, wrong convention in citing manuals and brochures, capitalization of publication names even though discouraged in the thesis guidelines)
- Some shortcuts are not introduced in the text and are not always used in a shortened version (°C) or even are not used in the text at all (e.g. CNS, E/M, HCV). The introduction of a shortcut is reasonable when used more than once in the text (RdRp).

Suggestions and questions, which the student has to answer during the defence.

1. Are there any additional functions attributed to C protein of TBEV or flaviviruses during infection pathogenesis instead of genome packaging?
2. Can you explain why is expression and purification of TBEV C protein so challenging? Why did you exclude C-anch from the thesis?
3. Can you explain what is the reason for using different temperatures for the protein expression with the same type of competent cells?
4. Compare the amplification conditions for PCR you used in tables 2-3, 2-5, 2-8 and explain the differences in the protocols used.
5. In figure 12 I am not sure whether you labelled the putative size of the C protein in the gel correctly. Could you provide the figure of WB that you omitted from the thesis?
6. Compare your best TBEV C purification results with a methodology used in a recent publication of Selinger et al., 2022 (https://doi.org/10.1016/j.jbc.2022.102585) and critically evaluate your protocol and suggest potential improvements.

Conclusion:

In conclusion, 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 the grade will be suggested after defence.

In **České Budějovice** 19th of January, 2023

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signature