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: Siroun Moura

Thesis title: Production and Biophysical Characterization of Phi8P4 Helicase

Supervisor: RNDr. Zdeněk Franta, Ph.D.
Co-supervisor: Mgr. Tomáš Fessl, Ph.D.

Referee: RNDr. Martin Selinger, 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	2
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	1
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
Graphic layout of the text and of the figures/tables	0-3	2.5
Quality of the annotation	0-3	2
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 labelling, indication of the units)	0-3	2
Formal requirements – points in total		13
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfilment of the aims	0-3	3
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	2.5

[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	1.5
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	1.5
Practical requirements – points in total		18
POINTS IN TOTAL (MAX/AWARDED)	48	31

Comments of the reviewer on the student and the thesis:

The bachelor thesis of Siroun Moura aimed to clone and optimize the production of P4 helicase protein of Phi8 bacteriophage (*Cystoviridae*) with subsequent testing of its activity *in vitro*. All goals were successfully fulfilled - clone with N-terminally His-tagged Phi8P4 was obtained and the large-scale production conditions were optimized as well. Moreover, the isolated Phi8P4 protein was proved to be functional using smFRET analysis. Thus the thesis represents a good piece of work with very interesting data.
The length of 41 pages (including the list of references and appendix) is more than satisfactory. The thesis is divided into eight chapters: Introduction, Aims, Materials and Methods, Results, Discussion, Conclusions, Refernces and Appendix. Following text will comment each of the chapters with questions asked after the comments.

Abstract
Abstract is very well written with just one minor mistake – the last sentence lacks the verb and thus seem incomplete.

Introduction
This chapter covers 12 pages with 8 figures, 2 tables, and 23 references being cited. The coherence of this part is relatively good, however, the incorrect syntax and generally bad language skills made the text quite challenging to follow. Many descriptions were quite chaotic and the frequency of grammar and spelling mistakes was quite high (especially a wrong use of singulars and plurals - eg. "An example of different types of viruses are mentioned below"). Except for wrong singular/plural forms I would like to further pinpoint the wrong form of noun/adjective (terminus vs. terminal).
Comments:
1) page 1 – no mention of enveloped viruses in the general description of viruses
2) I lack the information about Phi8P4 with C-terminal tag and the reason why the N-terminal tag is necessary. This in my opinion crucial for better understanding of the thesis' goals and therefore, it is quite unfortunate that this information is not mentioned until the discussion part.

Aims
Aims are clearly stated, I have no comments here.

Materials and Methods
This chapter covers 10 pages and is generally well written. I also highly appreciate the wide

spectrum of methods, which was used in the thesis.

Comments:

1) I don't understand, why Table III is not included in the chapter. Moreover, there is a considerable incoherence in the composition column of this table – you need to use either molarity in combination with mass/volume fractions or the mass and volume of the respective chemicals.
2) The origin of the cloning primers is not stated.
3) gradient PCR – the author states that the gradient PCR was performed using 45-65°C annealing temperature span. However, a different statement is written on page 26 (55-65°C).
4) Table VII: The final volume of the gel mixtures is missing.

Results

The results are described on 8 pages and are accompanied by 9 figures. Few grammar mistakes are present, but the chapter is well written with a good coherence and syntax.

Comments:

1) Images for gradient PCR and IMAC/SEC are missing. Please, see the questions.
2) The formulation on page 28 is probably wrong - "The highest amount of the protein of expected size ~35 kDa, was present in soluble fraction of *E. coli* cells upon induction with anhydrotetracycline and incubation at 18°C for 24 hours." I suppose that it should have been formulated like "the highest amount of Phi8P4 among samples from soluble fractions..." since there are quite high amounts of a ~35 kDa protein in insoluble fractions.
3) The procedure of protein concentration (mentioned in chapter 4.3.2) is not described. Please, see the questions.

Discussion

The discussion is quite short and I think that author did not understand how it should look like. Only 3 references are cited and the whole structure is just a summary of the methodology. I expected the comparison of the results with already published data and also a short reflection about the possible future plans.

Questions:

1) Based on an interesting nature of the *Cystoviridae* replication cycle, what antiviral mechanisms do bacteria actually posses?
2) What is the reason for studying the function of Phi8P4?
3) Why there is no image for gradient PCR? What was the concentration of the DNA template in the reaction? What was the range of the annealing temperatures?
4) Figure 12 – according to text the PCR product length is 963 bp. Is the author sure that the sample of interest is in the line 4, where the size of visible band is approximately 650 – 700 bp?
5) Author mentions protein concentration procedure in chapter 4.3.2. What was the procedure exactly? And which fraction(s) from SEC (Figure 17) were used for further experiments?
6) Why there is biotin anchored to the RNA hairpin?
7) Taking into account that you have a functional protein at disposal, are there any particular plans for the future research?

Conclusion:

The bachelor thesis of Siroun Moura, despite many formal mistakes, represents a nice piece of work, where the aspirant cloned and produced Phi8P protein, which was also proved to be fully functional. The obtained data will surely help in further research and will be a part of a scientific publication. Therefore, I **recommend** the thesis for the defense and the final grade will depend on the performance of the candidate during the defense and her answers to my questions.

In **České Budějovice** date **21.7. 2020**

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