



Přírodovědecká
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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: Nelli Manoczki

Thesis title: Mechanism of dsRNA virus replication: Cloning, production and structural characterization of C-terminal domain of σ NS

Supervisor: RNDr. Zdeněk Franta, Ph.D.

Referee: RNDr. Jindřich Chmelař, Ph.D.

Referee's affiliation: Department of medical biology, Faculty of Science, USB

	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	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	2
Graphic layout of the text and of the figures/tables	0-3	3
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	2,5
Formal requirements – points in total		17,5
(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	3
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	3

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

Experimental difficulty of the thesis, independence in experimental work	0-3	2
Quality of experimental data presentation	0-3	3
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		22,5

POINTS IN TOTAL (MAX/AWARDED)	48	40
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Comments of the reviewer on the student and the thesis:

Bachelor thesis of Nelli Manoczki focused on the construction of the expression construct with cloned C-terminal domain of a non-structural protein from Avian reovirus. Furthermore, the student attempted to express this protein in *E. coli* expression system. The thesis has appropriate length and structure, the chapters are well balanced. Overall, the thesis reads well, the amount of information is adequate and the graphical layout is good. There are, however, several points that needs to be mentioned that affect the work quality.

1. The English is sometimes incorrect, but this does not influence the understandability and readability of the work.
2. The number of references is rather low, especially the discussion lacks sufficient number of relevant citations.
3. Some figures have low resolution (e.g. fig 21) with worse readability of figure description

To individual chapters:

- Annotation and table of contents is good. Then, there are 2 lists of figures and tables, which I find rather unusual and I would not recommend the usage of this. However, I do not consider it a mistake.
- The introduction is logically structured; it describes the problematics in “general to specific” manner and goes from the classification of viruses to the description of the protein of interest.

Questions to introduction:

1. **Please show us the depiction of virus by ancient Egyptians, I failed in searching this online.**
2. **In 1.4.1. you write about using modified viruses in fighting cancer. Can you describe the proposed mechanism in more details?**

- Aims of the work are well defined.
- Materials and methods is a very nicely written, methods are described precisely with many details that would enable others to reproduce the experiments. I appreciate the use of concentrations everywhere (not only volumes), lack of which is often flaw of bachelor theses. The sequential description of amplification, cloning, expression and detection of protein by Western blot is clear and adequate. I have only 2 questions:

1. **How does the induction of expression by anhydrotetracyclin in the plasmid works?**
2. **Why did you use two different lysis buffers for pilot and large scale expression?**

- Results: The result section describes the progress in the preparation of the construct from the optimization of PCR to ligation into the expression vector, the expression itself (pilot

expression and large-scale expression) and the detection of expressed protein by western blot. The author unfortunately did not succeed in expressing desired protein in soluble form, only in inclusion bodies, but this does not diminish the quality of the bachelor work. The experimental progress is accompanied by figures that are mostly comprehensible, only figure 19 is hard to understand due to insufficient lane differentiation. I have several comments:

1. In chapter 4.1. you write that you obtained 618bp band. You cannot estimate the exact size of DNA on agarose gel.
 2. Please describe figure 19 during the presentation to show, what is what.
 3. The figure 22 shows 3 bands of degraded protein. Did you try to identify the individual bands?
- Discussion: Discussion is the weakest part of the work with only two references cited and 1 unpublished work. I think author should try to search more in the literature for possible solutions of degradation and expression in soluble form and could also suggest some future direction in pursuing this topic. I have only one question:
 1. What other way than proteolysis can lead to specific degradation of the protein as seen on the figure 22?
 2. According to known properties of the protein and the size of degraded fragments, can you predict the probable "cleavage" site?
 - References: The work contains 23 references, which is rather low number and especially in the discussion, there are only 2 references plus 1 unpublished. In the introduction there are parts, where the citation should be used. Moreover, most of the information on viruses is taken from the textbook Medical microbiology from 1996. I believe there are many recent reviews that could have been used. Otherwise, the references are used correctly in the same format throughout the work.

Overall, the work is experimentally sound with adequate introduction and proper description of methods and results. Considering negative results, the discussion should be prepared more carefully and definitely more references should have been used in both introduction and discussion, e.g. while discussing other possibilities of getting soluble protein or possible ways of degradation during the expression.

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade Very good (2) .²

In **České Budějovice** date 11.6.2019



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).