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: Trang Thanh Vu

Thesis title: Expression and Purification of Adhesive Recombinant Proteins, Sericin 2 and Salivary Gland Secretion 3
Supervisor: Prof. RNDr. Michal Žurovec, C.Sc.

Referee: Paulina Duhita Anindita, D.V.M., Ph.D.
Referee`s affiliation: Faculty of Science, University of South Bohemia in Ceske Budejovice

	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	2
Graphic layout of the text and of the figures/tables	0-3	2
Quality of the annotation	0-3	2
Language and stylistics, complying with the valid terminology	0-3	2
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		15
(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	2
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	2
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	3
Practical requirements – points in total		24

POINTS IN TOTAL (MAX/AWARDED)	48	(39)[2]

Comments of the reviewer on the student and the thesis:

The bachelor thesis written by Trang Thanh Vu presents an interesting work in an attempt to discover bioadhesive material which can be applied in biomedical field for instance. The thesis was written in a logical manner and Vu seems to understand the principle of experimental works presented in the thesis. Despite the challenging task to produce recombinant adhesive proteins, Ter1 and Sgs2, Vu managed to produce, purify and characterize the recombinant proteins. This thesis could be served as the basis knowledge for future works regarding development of bioadhesive materials derived from insects.

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

There are several points which should be addressed related to the thesis:

1. Be consistent with word spelling. Detailed proofreading would be useful to avoid such inconsistency.
2. What was the bacterial cell fraction (soluble/insoluble) run in the SDS-PAGE gel to examine the effect different induction times shown in Figure 2?
3. Why did the Western blot and Ponceau S staining in Figure 4 showed different results?
4. What was the band detected between 10 and 17 kDa protein marker in Western blot and Ponceau S staining in Figure 4, lane 9 (Sgs3 protein sample)?
5. As the production of soluble Sgs3 protein seems to be low in *E. coli*, is there any plan on optimizing the production using other expression system?
6. In Figure 9, the Sgs3 protein was not clearly visible as very thin bands correspond to 17.9 kDa were shown. How much confidence can be given to say that the Sgs3 protein was purified from the inclusion body?
7. The concentration of Sgs3 protein could not be determined from densitometry (section 5.2.4 andFigure 10). How the 0.5 mg/mL and 1.0 mg/mL of Sgs3 protein was determined for the cell culture experiments?
8. Although a negative control (BSA) was used during the cell culture experiment, why a positive control was not included in the experiment? It would be beneficial to also include a positive control, such as other recombinant adhesive protein with known adhesive properties for cell culture or a commercially available adhesive protein to support the findings as well as reducing a bias during observation.
9. In Figure 17, Ter1 protein coating on glass surface almost could not be seen but it was claimed to have a similar pattern with the result in Figure 16. How was it determined?

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[2] Enter the number of points awarded.

Mistakes, which the students should avoid in the future:

1. In general, figures containing SDS-PAGE gel and western blot pictures appear to be in lower resolution. The samples orientation presented in the SDS-PAGE and Western blot figures is not readily understood for the reader.
2. There are several mismatches between in-text citations and corresponding references in the *References* section. Several representative examples are below:
   a. The year of publication is written differently. This mistakes are mostly found in the *Introduction* section.
      p. 2: It was written as Weisman et al., 2009. However, the publication year written in the References section was 2010. (p.44: ... 2010. Honeybee silk: Recombinant protein production...).
      p. 2: It was written as Aramwit et al., 2011. However, it was written 2012 as in the *References* section (p.40: ... 2012. Potential applications of silk sericin...).
   b. Some in-text citations are missing in the *References* section.
      p. 37: ... (Bokner et al., 2016).
      p. 38: ... (Teulé et al., 2011).
   c. Some citations/references, either in-text or in the *References* section are not following the writing rules for references or simply having a typo.
      p. 4: ... (Gräslund et al., 2008). It was written as "Gräsland" in Gräsland, S., Nordlund, P... (p. 41, *References* section).
      p. 42: "desi42ahn42hesiveshesive" in Kord Forooshani, P., Lee, B.P., 2017. Recent approaches in desi42ahn42hesiveshesive materials inspired by mussel adhesive protein... (p. 42, *References* section).
3. "Competent cells" was mistyped as "component cells" (in *Table of Contents*, p. 7 and p. 9).
4. There are inconsistency in the *Method*s section.
   a. p. 9, section 4.1: "100 µL of these prepared cells were applied to an LB agar plate treated with ampicillin (final concentration 100 µg/mL)" and p. 9, section 4.2.1. "This medium was supplemented with ampicillin (final concentration of 0.1 mg/mL).". Please use either 100 µg/mL or 0.1 mg/mL.
   b. p. 12, section 4.7.2.: "... transfer to the nylon membrane ..." and "... the unstained gel were transferred to a PVDF membrane ...". It was not clear whether two different membranes used for Western blot experiment.

Conclusion:

In conclusion, I recommend the thesis for the defense and I suggest the grade 2.[3]

In **Ceske Budejovice** date 21 January 2020.

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signature

[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).