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: Stemmer Vitus

Thesis title: Production of recombinant proteins using prokaryotic and eukaryotic expression systems
Supervisor: RNDr. Ján Štěrba, Ph.D.
Co-supervisor: Kateryna Kotsarenko, MSc. Ph.D.

Referee: Pavel Grinkevich, Ph.D.
Referee`s affiliation: University of South Bohemia

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for master's 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	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
Graphic layout of the text and of the figures/tables	0-3	2
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	3
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	3
Formal requirements – points in total		20
(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

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

Completeness of the description of the used techniques	0-3	3
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	3
Practical requirements – points in total		25
POINTS IN TOTAL (MAX/AWARDED)	45	(0-48)[2]

The bachelor's thesis presented here by Stemmer Vitus deals with the search for the optimal production conditions of PNGase H^+ and the partial amplification of the genes that code for fucosyltransferase, α2,3-sialyltransferase α2,6-sialtransferase and DNA methyltransferases from *Ixodes ricinus*. Stemmer participated in all the stages of recombinant protein production, such as vector design, cloning and expression optimization. He also attempted to isolate the PNGase H^+ using affinity chromatography and test its enzymatic activity.

The thesis is clearly structured and well written in a mostly concise language that aligns well with the scientific writing standards.

There were a few typos and inconsistencies:

- Abstract, last line: *I. ricinus* is not italicized.
- P. 7, line 7 "The growth was": it is better to say "The culture was" or "The aliquot was".

Overall formatting remarks:

- The same font size and face as the main text was used for the page headers and footers, figures and tables descriptions which makes it a bit harder to read (a bit of nitpicking here, I admit).

Questions:

1. Are there any other explanations as to why the fragment for α2,3-sialyltransferase amplified in the confirmation PCR was much longer than expected?
2. Did you try to perform Western blot to confirm the presence of PNGase H^+ in the purified fractions?
3. Is the overall protein concentration in cell lysates a good indication of the production of a given recombinant protein?

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[2] Enter the number of points awarded.

Conclusion:

In conclusion, I recommend the thesis for the defense and I suggest the grade 1 .

Ceske Budejovice,
14 September 2020