Přírodovědecká fakulta
Faculty of Science

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

OPPONENT'S REVIEW ON DIPLOMA THESIS

Name of the student: Katharina Böttinger

Thesis title: Proteomic profiling of *Ixodes ricinus* tick cell line IRE/CTVM19 and its response to TBEV infection

Supervisor: Mgr. Dmitry Loginov, Ph.D.

Referee: Daria V. Vasina, Ph.D.

Referee's affiliation: A.N. Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences

	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	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	2
Formal requirements – points in total		19

	Point scale	Points
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	2
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	2
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	3
Logic in the course of the experimental work	0-3	2
Completeness of the description of the used techniques	0-3	3
Experimental difficulty of the thesis, independence in experimental work	0-3	3

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[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	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		24

POINTS IN TOTAL (MAX/AWARDED)	48	43

Comments of the reviewer on the student and the thesis:

Encephalitis as well as other tick-borne viral infections are currently of high epidemiological concern as they are a common cause of human viral brain infection and are capable to cause a wide spectrum of disease, ranging from asymptomatic to full-blown encephalitis or even death. The molecular aspects of pathogenesis process as well as transmission of the virus are of vital importance for practical purposes in terms of diagnosis, prevention and treatment of this disease. Thus, the subject of current research is of interest and can become the basis for future practical developments.

The strengths of the work include the use of high-precision methods, which allow to study the process of TBEV infection of cell culture of its main reservoir - Ixodidae ticks. This enabled to reveal a significant amount of proteins potentially associated with this process.

Of particular interest is the Principal component analysis provided during the study, which provided the cell biotyping of infection course, made it possible to identify a number of MS spectra peaks and to make their assignment with proteins identified by nanoLC-ESI-MS/MS. These results can be further used for *in vivo* studies with *Ixodes ricinus* as a part of development of a diagnostic system for encephalitis detection and disease's biomarkers search.

Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future:

Nevertheless, there are some concerns about the text of the thesis.

First, it is not evident from the text what was the aim of three *I. ricinus* cell lines comparative proteomic study using 2DE. There is no any further discussion of these results as well as no any premise for this experiment in Materials and methods section. The main idea of the thesis was to provide the comparison of infected and non-infected IRE/CTVM19 proteomes and it is not clear, how do proteomes of two other cell lines allow to achieve this goal. Moreover, the 2D profiles of the samples contained more than 700 protein spots while during the LC-MS/MS analyses of CTVM/IRE19 cells only 61 and 280 proteins were identified on days 2 and 10 respectively. In this regard, one can assume that proteome profiling with 2D electrophoresis is a method of choice for this type of study.

Second, the aim of the study should be formulated more precisely: a comparative proteomic analysis itself is just a mean of achieving the goal, and the goal could be, for example, to identify markers of infection, to study the process of TBEV pathogenesis, to find out the metabolic changes of the host in the presence of a virus, etc.

Conclusion:

In conclusion, I recommend the thesis for the defense and I suggest the excellent (1) grade.[2]

In date *14. 01. 2019*

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signature

OPPONENT'S REVIEW ON BACHELOR/DIPLOMA* THESIS

Name of the student: Bc. Katharina Böttinger, BSc.

Thesis title: Proteomic profiling of *Ixodes ricinus* tick cell line IRE/CTVM19 and its response to TBEV infection
Supervisor: Mgr. Dmitry Loginov, Ph.D.

Referee: RNDr. Jindřich Chmelař, Ph.D.
Referee`s affiliation: Department of Medical Biology, 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	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	3
Quality of the annotation	0-3	2
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	2
Formal requirements – points in total		19

(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

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*   Choose one

[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	3
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		26

POINTS IN TOTAL (MAX/AWARDED)	**48**	**45**

Comments of the reviewer on the student and the thesis:

The work of Katharina Böttinger deals with mass spectrometry proteome analysis of the tick cell line IRE/CTVM19 in response to TBEV infection. The work has 49 pages, including the list of references plus 7 pages of supplementary tables.

The thesis contains detailed list of abbreviations and table of contents. The annotation itself is rather short, could be few sentences longer to describe the work in more details. On the other hand, it summarizes the thesis in a single sentence, so it fulfills the purpose of annotation. The work itself begins with an abstract, which is clear an nicely written.

The work itself is, in my opinion, excellent from all points of view (formal, content, readability, logical structure, work with literature, broad range of experimental approaches, precise description of methods). The only points, I have to mention, are the length of annotation and the lack of figures in the introduction (point 1 bellow). The last issue I have to mention is the lack of the discussion of the significance of the results. What the results tell us about the difference between controls and infected cells from the functional point of view, e.g. what cellular processes are affected and can it have some implication in the control of virus transmission? These questions could have been developed in more details in the discussion. The discussion is combined with the results, which is ok with me.

I must point out excellent work with the literature, especially in the introduction, which is perfectly structured, with high number of references, so that the reader is lead from general to specific. It is obvious that the author understands very well the topic to details and can describe it even to the reader out of her research field.

In addition, the description of the methods is very precise and detailed and the author understands the principles of the methods.

Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future:

Suggestions and comments:
1. Introduction would benefit from more figures, especially when specific signaling pathways are described, it is difficult (at least for me) to follow the pathway without graphical scheme.
2. I am not sure, if this is really mistake, but usually, it is recommended to use "author, date" style of citations and alphabetical order of references instead of number style in

diploma theses at Faculty of Science.
3. Despite otherwise excellent written language, I found several missing commas.
4. The nature of numbers, used for protein assignment, should be mentioned first time, they are used (in the main text, page 24). It is described only in the tables that these are accession numbers to UniProt database.

Questions:
1. At page 6 and 7, the author describes available tick cell lines and that the proteome of morphologically distinct cell lines (i.e. ISE6 and IDE12) is identical. How is this possible?
2. I did not find the type of cell culture plates (i.e. how many well plates were used) in the chapter 3.2. Please describe
3. I did not find the process of how GO analyses were done. Please describe.
4. What is the principle of guanidination derivatization of peptides? Does it work also in the complex protein mixtures, such as serum?
5. At page 28, author mentions that there was a difference 1 amino acid, suggesting these are homologues. Could it be individual polymorphism or some kind of sequencing artefact?
6. Can the found differences between control and infected cells be used for TBEV targeting?
7. Is it possible to compare your results with some proteomic comparisons of mammalian dendritic cells or other mammalian cell lines infected with TBEV, i.e. is there some similarity in affected enzymes?

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade   1 - excellent   .[2]

In   **České Budějovice**   date   **14.1.2019**

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signature

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[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).