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: Barbara Jetzinger

Thesis title: Proteomic analysis of hemolymph during immune response of *Drosophila melanogaster* larvae by UPLC-MS

Supervisor: doc. Mgr. Tomáš Doležal, Ph.D.

Referee: Mgr. Dmitry Loginov, Ph.D.

Referee`s affiliation: 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	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	1
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	1
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	1
Formal requirements – points in total		13

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

(2) PRACTICAL REQUIREMENTS

Clarity and fulfillment of the aims	0-3	1
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	1
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	1
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
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	0
Practical requirements – points in total		12
POINTS IN TOTAL (MAX/AWARDED)	48	25

Comments of the reviewer on the student and the thesis:

You should be more careful in the data interpretation

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

Suggestions:

1. The introduction part should be in agreement with the thesis title. The state of art in *Drosophila* hemolymph proteomics should have been provided.
2. Sufficient details should have been provided in the methodological part to allow the work to be reproduced, e.g. amount of added enzymes, not only their concentrations. Also, all steps of optimization must be described.
3. The results should be presented in a proper way. For proteomics it is necessary to provide such parameters as score, number of peptides,

sequence coverage, FDR, etc. to show your identifications are reliable.

4. The discussion part should be more dedicated to your results, than description of other studies. You should have discussed your results in comparison with other proteomic studies to specify why you work is important. Also, you should have been more focused on the comparison of the whole protein profiles of infected and uninfected sample, e.g. you could use Gene Ontology analysis for it.
5. The language should be improved to avoid not only grammar mistakes, but also misusage of terminology (e.g. A further explanation for proteins not found during our analysis are the limitations in protein quantification; hemolymph vs plasma) and incorrect statements (It is important to stress that this work represented the first use of proteomic analysis in the laboratory and the methodology had to be optimized). Latin names of organism must be written in italic style.

Questions:

1. Not a word is written in material and method section related to the optimization. What exactly was optimized?
2. Why did you omit the steps of reduction and alkylation before protein digestion?
3. How the "sediments" were processed and why did you need to do it? It was not described in material and methods.
4. Why did you use 2 databases: NCBI and UniProt?
5. Were the sequences of internal standards added to the database? Can you explain the reason of their degradation so you were not able to detect any peptides?
6. The fold changes of protein expression between experiments 1 and 2 were explained by different conditions of sample preparation. Then, why did you call them "Biological replicates"? How could the different conditions (freezing) change the results so much?
7. In the table 1 you presented proteins with significant differences. In the table 2 – proteins with notable trends. What did you mean by word "significant"? Please specify which notable trends did you observed.
8. In the tables you wrote that for some proteins "Zero abundances were detected in uninfected samples". Can these proteins be considered as protein markers of wasp parasitoidism?

9. You discovered a decreased level in supernatant and an upregulation in sediment of the protein HDC10707, and suggested an alteration of its chemical properties upon infection. What alterations did you have in mind?
10. You found wasp proteins in infected and control samples, and explained it by the limitations of the analysis. Did you verify if there were any possible alternative/homologous proteins belonging to the *Drosophila melanogaster* instead of *Leptopilina boulardi* in the control sample?

Conclusion:

In conclusion, I

recommend / ~~do not recommend~~*

the thesis for the defense and I suggest the grade 3 .[2]

In ČB date 29.08.2018

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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).