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: Nora Andrea Hagleitner, BSc.

Thesis title: Transposon mutagenesis in the Lyme disease pathogens *B. afzelii* and *B. burgdorferi*

Supervisor: Ryan O. M. Rego, Ph.D.

Referee: RNDr. Helena Langhansová, Ph.D.
Referee`s affiliation: Dept. 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	2.5
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	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	2.5
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	2.5
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	2.5
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	3
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		25
POINTS IN TOTAL (MAX/AWARDED)	48	45

Comments of the reviewer on the student and the thesis:

The submitted work has numerous aims and several sub-projects indicating that the student had to deal with many methods and did impressive piece of work. I appreciate especially the long-term cultivation of spirochetes where one mistake in aseptic conditions usually means the loss of several-week work. Moreover, experiments based on the vector–host–pathogen interactions are always tricky since all three biological entities (ticks, mice and *Borrelia* in this case) often live their own lives regardless researcher´s wishes. Although it is clear to me that the fragmentation of the thesis originates from several projects being currently investigated in the supervisor´s lab, it was sometimes hard to keep reading and not to lose connection.

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

1. Formal mistakes: Most spelling mistakes (although sporadic in general) are in pretty important terms: l. scalpularis, erythema migraines etc. The format of citations in the list is not uniform. As far as I can judge, two-word terms should be written with hyphen: arthropod-borne, tick-transmitted, site-directed etc. In Fig. 12 and corresponding text, there is a mistake in units; I guess it should be µm instead of mm.
2. p. 1: "...Only 3 genospecies of the *B. burgdorferi* sensu lato complex are responsible for human cases of Lyme disease..." – single cases of human Lyme borreliosis were presumably caused by other genospecies. Could you please mention them?
3. p. 10: What does "biphasic dissemination of spirochetes" mean?
4. p. 14: How were primary antibodies for western blot prepared?
5. Some results (e.g. chapter 4.2.8.4 Microdilution assay) are described only verbally without any figure, table or graph. Similarly, no microphotograph from fluorescent microscope documents the work with fluorescent mutants. Even if the results are negative, a few pictures could nicely supplement the thesis.
6. p. 51: "presence of gentamycin gene" would be more exact formulation.

Conclusion:

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

In České Budějovice, January 15th, 2019

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RNDr. Helena Langhansová, Ph.D.

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OPPONENT'S REVIEW ON DIPLOMA THESIS

Name of the student: Bc. Nora Andrea Hagleitner, BSc.

Thesis title: Transposon mutagenesis of the Lyme disease pathogens *B. Afzelii* and *B. burgdorferi*

Supervisor: Dr. Ryan O. M. Rego, PhD

Referee: Mgr. Jaroslava Lieskovská, PhD

Referee`s affiliation: Department of Medical Biology, Faculty of Science, University of South Bohemia, České Budějovice

	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	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		21
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	2.5
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	3

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

Logic in the course of the experimental work	0-3	3
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)	47

Comments of the reviewer on the student and the thesis:

Thesis written by Nora Hagleitner deals with the production and functional characterization of transposon mutants of various species of borrelia spirochaetes with aim to identify genes important for infectivity of this pathogen. Student performed the functional characterization of three transposon mutants of *B. burgdorferi s.s.* (B31-A3) by determining their growth rate, motility, morphology and infectivity. Especially one of the mutant named as [redacted] appeared to be interesting with sever effects on tested parameters, including infectivity. The attempt to create transposon mutants on infectious *B. afzelii* background was also undertaken and several other tasks were solved. This high quality thesis thus consists of many partial aims which are parts of research project of Dr. Rego. I would like to emphasize the broad scale of methods which were used in the thesis and the amount of work which was done. I believe that student significantly contributed to the solving of the project. The thesis itself is very well written with clear description of research topic in introduction, methods and results. These are followed by discussion which is confined to the covering obtaining results and in addition it offers the possible reasons for negative outcomes and outlines the direction of future research.

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

1. Page 44, you wrote that *I.ricinus* is not the natural arthropod tick vector for *B. burgdorferi s.s.* This is not correct statement. Although *B. burgdorferi s.s.* is not the major spieces trasmitted by *I. ricinus* it is still naturally transmitted by this tick.
2. To add the paragraph into discussion about the connection between infectivity of borrelia and flagelly assembly.
3. To add a list of abbreviations which is missing in the thesis.
4. To use the word ‚intraperitoneally' instead of ‚intraperitonedly'.

Questions:

1. Page 1, you stated that Lyme disease can be caused only by three genospieces of *B. burgdorferi* sensu lato complex, by *B. burgdorferi* s.s., *B. afzelii* and *B. garinii*. There are other spieces of borrelia which were isolated from patients suffered from Lyme borreliosis. Please correct your statement.
2. Page 19, why were plates incubated in the presence of CO_2 when determining the limiting dilution of borrelia transformants? CO_2 is usually not required for culturing bacteria.

3. Page 38, you mentioned that cp9 is missing in *B.burgdorferi* B31. Is it known what function has the gene encoded by this plasmid? Could it impact transposon mutagenesis?
4. Page 40, you showed the morphology of transposon mutant [redacted] and concluded that there is no change when compared to wild-type B31. It seems to me that there is a visible difference. Could you please comment it.
5. What was the rational for chosing in some experiment three different strain of mice, Balb, C3H and Ggtal? Could you provide some short characterization of these mouse strains in relation to borrelial infection?
6. Would you have some explanation why where was the transposon borrelia mutant [redacted] with decreased infectivity detected just in ears? Is it common when doing such infectivity experiment to find it in this place? If yes, why?
7. What other factors (in addition to one causing changes in morphology and motility) could influence the infectivity of borelia?
8. Mutant [redacted] as disruption in [redacted] ne locus. You first considered the [redacted] to be a product of this gene due to the presence of [redacted] domain. Based on phylogenetic analyses, comparison of 3D structure, and missing effect on cell wall integrity you concluded that it is not [redacted] Have you done the visualization of 3-D structure using Chimera programme by yourself?

Mistakes, which the students should avoid in the future:

Use list of abbreviations.

Conclusion:

In conclusion, I r e c o m m e n d the thesis for the defense and I suggest the grade 1.

In **České Budějovice** date **14. 1. 2019**

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signature