



Přírodovědecká
fakulta
Faculty
of Science

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

OPPONENT'S REVIEW ON BACHELOR/DIPLOMA* THESIS

Name of the student: Bc. Anna-Celine Dankmaier, Bsc.

Thesis title: Differentiating the Adhesion Properties of the Surface Proteins between Various *Borrelia* species

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

Referee: Mgr. Jaroslava Lieskovská, Ph.D.

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	2,5
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	2,5
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		18
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	1,5
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

* Choose one

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

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
Quality of experimental data presentation	0-3	2,5
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	2
Practical requirements – points in total		23

POINTS IN TOTAL (MAX/AWARDED)	48	41
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Comments of the reviewer on the student and the thesis:

The thesis written by Anna Danklmaier deals with the function of Decorin binding proteins A and B of various *Borrelia* species, an etiologic agent of Lyme disease. DbpAB proteins are adhesins which are important determinants of infectivity since they influence the colonization and dissemination of borrelia as was learned from studies using *Borrelia burgdorferi* s.s. The role of DbpAB in European strains of borrelia is largely unknown therefore the chosen topic is important. Particular aims of the thesis were the creation and characterization of DbpAB overexpressing mutants in *B.afzelii* CB43 and creation of plasmids carrying *DbpAB* genes from various European borrelia strain to be expressed in DbpAB deficient strain of borrelia BbKH500. This genetic approach is attractive but also quit challenging since the transformation of borrelia may results in losing intrinsic borrelial plasmids. Student used a number of methods of molecular and cellular biology. Within first task, a few clones were obtained and tested, however the functional tests were limited to Swarm motility assay showing no differences. The creation of plasmids carrying DbpAB of various Borriela was attempted using three different expression plasmids but not completed. In spite of prevailed negative results I think the contribution of student to the project valuable and should be appreciated. I like also the phylogenetic analysis of DbpA comparing different European and American strains of *Borrelia* which is included in the thesis.

The thesis is written by good english though occasionally some stylistic could be improved. The literature overview consists of an information about borrelia, genome composition and transmission. The extra chapter is devoted to adhesion molecules including the role of Decorin binding proteins of various borrelia species in relation to dissemination and infectivity of borrelia; this chapter is quite extensive and less clear. Introduction is properly supplemented by figures,

from informative aspect the literature overview is sufficient. The aims should have been defined better. Since more borrelia strains were used in the thesis the information about origin of *DbpAB* gene in shuttle vector would be beneficial. Aim three was not addressed in the thesis. Methods are very well described allowing the repetition of all experiments. As concern the chapter results, it is written clearly with some exceptions. Information about plasmid profiles are summarized in two tables and indicates which plasmids were lost during transformation. It is pity that a proof about overexpression of *DbpAB* is missing though effort was done to prove it. Discussion is properly written with respect to content and length discussing results and possible reasons of not obtaining constructs.

Minor comments:

1. The information about the origin of used material is not always complete. For example:
Page 24 The source of rabbit anti-*DbpA* antibody, some strain of borrelia and secondary Ab used in WB are missing
2. There were a few not accurate formulation. For example:
Page 23 ...'since the immunolocalization assay did not cause any labelling of the mutant *DbpA* '.....
Page 34'It was found that the anti-rabbit *DbpA* of *B.afzelii* A91 is not able to bind to a CB43 lysate' (you probably meant rabbit anti-*DbpA* antibody, which recognizes *DbpA* of *B.afzelii* A91, is not able to bind to *DbpA* of *B.afzelii* CB43)
3. Small mistakes are present throughout the thesis.
Page 20...primary antibody should be rabbit anti-*DbpA* instead of anti-rabbit-*DbpA*, same mistake appears through all thesis (in pages 22, 23, 34,..)
Picture 4.1.2 labeling ,PBr' is used as antibody and then as cell lysate from *B.garinii*; there is a need to distinguish it.
Page 32... line 4 should be Table 4.1.3 instead of 4.1.2.
Page 1... The information about genome size of borrelia is not clear due to usage of not correct units (kbp versus bp)
4. List of abbreviations is missing.

Recommendation:

There are a lot of combination of cloning, rescuing and complementation involving various strains of *Borrelia* therefore more informative labeling of vectors or clones would increase the orientation throughout the thesis. For example, in aim 2 it would be clearer for readers if aim 2.2 would be ,Creation of shuttle vector expressing *B. afzelii* *DbpAB* (SV2G-*DbpAB*-Ba), shuttle vector expressing *B. garinii* *DbpAB* (SV2G-*DbpAB*-Bg), and shuttle vector expressing *B. bavariensis*

DbpAB (SV2G-DbpAB-Bb).

Questions:

Q1 Results from Swarm motility assay are presented in Figure 4.1.3. I do not see the correlation between numbers provided in text (related to an average diameter of grown colonies) and what is shown in the graph. Could you please explain and clarify what bars in the graphs mean?

Q2 On page 35 you write that both mutant A4 and CB43/26 grow at a very similar rate. It seems to me that growth is delayed. Was there no statistical significance reached at any timepoints? Was experiment repeated?

Q3 Page 34. The proof that DbpA is overexpressed in created CB43 mutants is necessary. Since antibodies recognizing DbpA failed (I guess same antibodies were used in ELISA and immunolocalization assay) would you think about another method to confirm the overexpression of tested genes?

Q4 In aim three you planned to create the suicide shuttle vectors. In conclusion you have written that creation of European DbpAB was attempted but not finished. Throughout the thesis there was nothing written about this. How does the system work? And do suicide shuttle vectors differ for dbpAB in *B. afzelii* and *B. bavariensis*?

Q5 Decorin binding proteins A and B (DbpA and B) of Lyme borreliae are of critical importance for the virulence of the spirochetes since their adhesion to host tissues could significantly impact the dissemination and colonization. Can you provide some simplified relationship between presence of specific proteins (substrate) in ECM (in skin, joint), expression of Dbps of individual Lyme borreliae and their infectivity?

Conclusion:

In conclusion, I recommend the thesis for the defense and I suggest the grade 1-2.²

In České Budějovice date 21. 1. 2022


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).



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OPPONENT'S REVIEW ON DIPLOMA THESIS

Name of the student: Anna-Celine Danklmaier

Thesis title: Differentiating the Adhesion Properties of Surface Proteins between Various *Borrelia* Species

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 scale¹ 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	2.5
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Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	2.5
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	1.5
Formal requirements – points in total		18.5

(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	2.5
Logic in the course of the experimental work	0-3	2.5
Completeness of the description of the used techniques	0-3	3

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Experimental difficulty of the thesis, independence in experimental work	0-3	3
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	1.5
Practical requirements – points in total		18.5

POINTS IN TOTAL (MAX/AWARDED)	48	37
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Comments of the reviewer on the student and the thesis:

Briefly, Anna has submitted master thesis with very detailed literature overview, very (maybe too) challenging aims and precisely described methodology (although I miss primer sequences). My reproaches go mostly on how the results are presented and interpreted (see below). There is no doubt that European *Borrelia* strains/isolates deserve at least the same attention as their American fellows. In addition, I am not going to dump on the student because of the procedures that did not work or experiments that did not give the desired results since it is reality in research and I believe Anna worked hard. However, I felt puzzled even after several readings of result section. Among others, the first paragraph on p. 32 is misleading and confusing in many aspects (wrong table numbering, number of tested clones in the text does not correspond with the data shown in Tables 4.1.1 and 4.1.2, etc.). Other ambiguities are referred later within questions 2 and 3. Additionally, statistically significant results should be marked in the graph (Fig. 4.1.4).

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

Mistakes, which the student should avoid in the future:

Q1: How often does Lyme disease manifest as acrodermatitis chronica atrophicans? Although *B. afzelii* is the most prevalent LD agent in central Europe, I do not know anybody with this disorder (in contrast to several Lyme arthritis and neuroborreliosis cases among my friends and family).

Q2: As for expression of DbpA, why did not you try real-time qPCR to quantify *dbpA* transcripts in mutant *Borreliae* (instead of rather unsuccessful protein detection by three different immunomethods)? What was the origin and clonality of the primary antibody used in ELISA/WB? Why did you use this ELISA setup with direct coating of measured antigen instead of coating of capture antibody? Could you please share some raw data (absorbances) showing that different dilutions of coated lysate are visibly distinguishable (p. 21)?

Q3: I am definitely not an expert in western blotting, but I cannot see any band around 20 kDa (predicted MW of Dbp, p. 9) in Fig. 4.1.2. What does precisely mean if "lysate works in western blot"?

Q4: The main function of Dbp seems to be the binding to ECM components during dissemination in the host. However, there is no such substrate in your swarm motility assay. How could the growth in liquid medium be affected by Dbp overexpression? Do you know any *in vitro* test where motility within ECM can be measured?

S1: Although very few typos are present in the thesis, next time please double check units (bp vs. kbp, p. 2), automatic corrections (gene conversation, p. 3), citation manager job (p. 10) and Latin spelling (*Borrelia*, Tab. 3.3.1 and Fig. 4.2.1).

S2: List of abbreviations might be useful for some readers.

Conclusion:

In conclusion, I r e c o m m e n d the thesis for the defense and I suggest the grade “very good” (2).

In České Budějovice, January 12, 2022

A handwritten signature in blue ink, appearing to read 'Langhansová', with a small '1' written above the end of the signature. Below the signature is a horizontal dotted line.

RNDr. Helena Langhansová, Ph.D.

