



Přírodovědecká
fakulta
Faculty
of Science

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

SUPERVISOR'S STATEMENT ON BACHELOR THESIS

Name of the student: Sabrina Vranjes

Study program: Biochemistry

Department/Institute: Institute of Parasitology, Academy of Sciences of the Czech Republic

Thesis title: Differential expression of genes in replicative (spiral) and persistent (biofilms) forms of the causative agent of human Lyme disease.

Supervisor: MSc. Maryna Holovčenko

Supervisor's affiliation: Scientist, Laboratory of Molecular Ecology of Vectors and Pathogens

Co-supervisor, consultant: Rudenko Natalie, PhD

	Point scale ¹	Points
(1) FORMAL REQUIREMENTS		
Formal and graphical quality of the thesis	0-3	3
Ability to work with literature	0-3	3
Language and stylistics	0-3	3
Formal requirements – points in total		9
(2) PRACTICAL REQUIREMENTS		
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 results and their discussion with the literature	0-3	3
Experimental difficulty of the thesis, independence in experimental work	0-3	3
Contribution of the thesis to the knowledge in the field and the possibility to publish the results (after eventual supplementary experiments)	0-3	2
Practical requirements – points in total		14
POINTS IN TOTAL (MAX/AWARDED)	23	(0-24)²

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

² Enter the number of points awarded.

Comments of the supervisor on the student and the thesis:

The bachelor thesis of Sabrina Vranjes aims to study the differential expression of genes in replicative (spiral) and persistent (biofilms) forms of the causative agent of human Lyme disease. The analysis was performed with the aim to determine the genes ever-expressed in biofilms and down regulated in spiral forms of borrelia. Sabrina's work is the small part of the bigger project, the main purpose of which is the establishment of the markers that will enable to detect "hidden" borrelia forms in the patients with chronic Lyme borreliosis in which the standard tests failed

After initial supervision, Sabrina managed to work independently, with great responsibility and care. The initial goal of Sabrina was to master the method of the formation of Borrelia biofilms by triggering it in growing cultures of spirochetes. It must be mentioned that this goal was not an easy one as not all borrelia cultures are "eager" to form biofilms *in vitro*. The credit should be given to Sabrina, she succeeded in this assignment and managed not only to grow a lot of borrelia biofilms but also to document the process of their formation. Selection of the appropriate "biofilm" markers and their analysis was conducted using different laboratory methods of molecular biology that Sabrina successfully learned and implemented in her research. The following techniques were involved in the project: preparation of media for borrelia cultivation, cultivation of borrelia spirochetes, triggering the biofilms formation, dark-field microscopy, DNA, RNA isolation, cDNA synthesis, PCR primers selection, PCR, nested PCR, RT PCR, gel electrophoresis, PCR products purification, direct sequencing of PCR products, cloning, and transformation. The project itself involved a lot of experiments and repetitions that Sabrina successfully fulfilled. She established the optimal conditions for PCR detection of biofilm forms of LD spirochetes and found two genes that could be used as the „biofilms markers“, i.e. the genes that are up-regulated in biofilms and down-regulated in spiral forms of *tested Borrelia*. At the final stage of her work, Sabrina has shown that she can work with internet databases as well as with scientific literature. She wrote a detailed but at the same time very specific introduction to her work and interpreted the obtained results in comparison with previously published data.

Despite the limited time given and a lot of experimental work, as well as restrictions caused by COVID epidemic, the results obtained by Sabrina are of scientific importance and can be considered as the starting point in the further investigations of the form-specific markers for human chronic LD, cause, most probably, by borrelia atypical forms.

Besides the research, I would like to mention that Sabrina is a pleasant, easy going, opened and helpful person. I am sure her presence will be appreciated by any lab.

Conclusion:

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

In České Budějovice

14.06.2023