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: Simon Halas

Thesis title: Expression, purification, and crystallization of the putative transcriptional regulator MSMEG_6227 from Mycobacteria.

Supervisor: RNDr Roman Tůma, Ph.D

Referee: Ryan O. M. Rego Ph.D.

Referee`s affiliation: Institute of Parasitology, BC, CAS & Faculty of Science, USB

	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	2
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	2
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		17
(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	2
Logic in the course of the experimental work	0-3	3

* Choose one

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

Completeness of the description of the used techniques	0-3	2
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	2
Practical requirements – points in total		22
POINTS IN TOTAL (MAX/AWARDED)	**48**	**39**

Comments of the reviewer on the student and the thesis:

The thesis by Simon Halas which describes the methods used in solving the aims of expression, purification and crystallization of the putative transcriptional regulator MSMEG_6227 is well organized and overall well written. The student has very clearly followed the rules in writing the thesis and for some limited mistakes which I point out in the section below is above par for a Bachelor's thesis.

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

Questions to be addressed at the time of the defense:

1. MSMEG_6227 has already been shown to have a homologue in *M. tuberculosis* (Rv3488) albeit at 40% similarity . The crystal structure for this particular protein has been elucidated (Citation 29 in the thesis) and was also expressed in *M. smegmatis* to show its functional role as a transcriptional regulator. Given the work that has been already carried out, I would like to understand how the crystal structure of MSMEG_6227 from *M. smegmatis* will enhance efforts against *M. tuberculosis* as pointed out in the introduction.

2. It was upon sequencing, seen that the S2-R105 deletion sequence contained a Serine to Proline mutation in the C-terminal domain. Can the student describe what should have been the right step to take after this mutation had been identified upon sequencing?

3.Also the student went ahead with using the mutated sequence above as they say it was not in the DNA binding region and not thought to be functionally relevant (Page 35). Yet in the proposed steps to be undertaken in the future, one of them is to obtain a sequence without the Serine to Proline mutation. What would be the need for this if this mutation is not thought to be affecting the function of MSMEG_6227

4. The student suggests, that in the future, optimization of the expression and purification procedures need to carried out for the full length version of MSMEG_6227. This was the initial aim of this thesis. I would like to know what could be changed and why was this not done during the duration of the work.

5. Section 3.4 Western Blot. For the vertical transfer to happen can the student explain in what order are the filter papers, membrane and gel kept and would there have been a better way for him to describe this in this section of the methods? Also in this section is it tween 20 or 80 that is used? Could he please provide the composition of the blocking buffer, which I could not find in this section? Finally please note that the bound antibodies are 'imaged' and not 'imagined' as written in the thesis.

6. So as to maintain the score of the experimental difficulty and also the grade for the

overall thesis, I would like to ask outside of the microscopy work that was undertaken, were all the other methods stated in this thesis carried out by the student?

Minor comments/suggestions

The student does need to be careful of some specific errors when writing a thesis of their work

1. Page 11- Section 3.1, just mentioning that the tube was kept on ice is sufficient and not needing to say -4C. The same section the full form for SOC needs to be stated. Also in this section the concentration of ampicillin in the agar plates needs to be stated. Section 3.2 – last sentence on Page 11, a bacterial culture is just that and stating 'the mixture containing the bacterial culture....' makes no scientific sense.

2. Section 3.2 It is Laemmli buffer and not Laemmle.

3. Page 22 It should say after 6 days and after 4 months.

4.PCR conditions can and are usually stated as a sentence and there is no need for a table of these conditions , which takes up a lot of written space.

5. Although all the protein gels had the sizes of the bands for the ladder stated well enough except for Figure 22, none of the figures in the thesis having the DNA gels had the bands of the ladder pointed out except for 1-2 bp sizes. This is definitely not the kind of images that should be used in a thesis or for publication.

6. Page 43 Paragraph 3 it should be Trutneva and colleagues, not Co which stands for company.

7. The vector map provided at the end is not useful for the reader (it is a readily available map online) unless it had some sort of demarcations to where the cloned pieces of DNA were inserted.

Conclusion:

In conclusion, I

r e c o m m e n d / ~~d o n o t r e c o m m e n d~~*

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

In **Ceske Budejovice** date **13/9/21**

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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).