OPPONENT'S REVIEW ON BACHELOR/DIPLOMA[*] THESIS

Name of the student:

Thesis title: Characterization of Photosystem I in the Red Alga Porphyridium purpureum

Supervisor: RNDr. David Bína, PhD

Referee: RNDr. Erica Belgio, PhD
Referee`s affiliation: Institute of Microbiology, CAS, Opatovicky mlyn, 379 81 Třeboň, ČR.

	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	0
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	0
Quality of the annotation	0-3	2
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	0
Formal requirements – points in total		8
(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
Completeness of the description of the used techniques	0-3	0

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

Experimental difficulty of the thesis, independence in experimental work	0-3	1
Quality of experimental data presentation	0-3	0
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		16

POINTS IN TOTAL (MAX/AWARDED)	**48**	**24**

Comments of the reviewer on the student and the thesis:

The thesis reports on the purification, biochemical and spectral analysis of Photosystem I supercomplex isolated from a mesophilic algae (*P. purpureum*). The subject is of great interest and the methodologies applied appropriate but some drawbacks undermine the importance of the results obtained. The description of methods is generalistic, the most relevant experimental details (detergent solubilisation, chlorophyll concentration, etc.) are missing; figure presentation is poor. In the Results ans Appendix sections axes and legends are wrongly labelled or missing, thus making data interpretation difficult for the reader and giving an overall impression is of inaccuracy. However, it must be recognised that the experimental work lead to the successful purification of Photosystem I, for the first time from this type of algae; it therefore constitutes a potentially valid contribute to the photosyntetic literature. For this reason, despite the limits, I recommend the thesis for the defence, with the advise to re-write it in the future.

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

Suggestions: 1) add page numbers; 2) substitute Figures with higher resolution ones; 3) check carefully correctness of X and Y axes and Figure legends; 4) implement the introductory part with i) algal phylogenesis; ii) photosystem I bioenergetics, in particular in relation to the time-resolved measurements presented in the results session; 5) make sure that the solubilisation steps are correctly explained, especially the detergent to chlorophyll ratio; 6) make sure that all the data presented in Appendix are cited in the text; 7) reference session should be implemented (for example, for equation 1.1, the original work by Plank should be cited).

Questions:

Q1: Motivate reasons for "lack of environmental control" in growth conditions (session 4.1). What is the meaning of "spectrum of *P. purpureum* culture upon time of the harvest"?

Q2: Section 4.2: was ultracentrifugation done on the cells of *P. purpureum*?

Q3: In relation to the previous point, explain the reasons why it was necessary to quantify chlorophyll concentration (as mentioned in Section 3.1) before sucrose density gradient fractionation.

Q4: Is X axes of Figure 13 correct?

Q5: Please explain better what it fraction 4a (in ml) from anion exchange chromatography.

Q6: Session 4.5, which fraction from anion exchange was exactly used for size exclusion chromatography?

Q7: Session 4.6, was HPLC analysis done on the intact PSI supercomplex or did you perform a

pigment extraction step before? Please, describe it.

Q8: Session 4.7, spectroscopy. Suggestion: it would be more informative to present in Figure 17 (left) also a low temperature steady state emission spectrum of the complex.

Figure 17 (right), it would be useful to show for comparison also the (slow) decay of an antenna complex.

Q9) Why do you think that 30 ps can be "straightforwardly ascribed to excitation trapping"? Please, dedicate a paragraph to this (Photosystem I bioenergetics) in the introductory session and also in the discussion.

Q10) Session 4.7.2 time-resolved absorption spectroscopy is a highly advanced technique that requires deeper knowledge and explanations. Please, consider removing part or all of it, focusing on improving what has already been presented. Conclusions should then be revised in line with this.

Conclusion:

In conclusion, I (recommend) **do not recommend***

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

In   **21.01.2020**   date

Erica Belgio

signature

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[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).