Prof. RNDr. Josef Komenda, DSc.
Institute of Microbiology
Academy of Sciences of the Czech Republic
Department of Phototrophic Microorganisms
Opatovický mlýn
379 81 Třeboň
Czech Republic

Review of the Master thesis entitled:
" Light harvesting complexes and chromatic adaptation of Eustigmatophyte alga Trachydiscus minutus" by Marek Pazderník

The aim of the presented thesis was to optimize method for separation of photosynthetic membrane complexes from the eukaryotic alga *Trachydiscus minutus* and using this method to explain mechanism of chromatic adaptation occurring in this organism.

Introduction contains description of principles of photosynthetic energy transformation with emphasis to that occurring in eukaryotic chloroplasts. It includes properties of photosynthetic pigments, mechanisms of charge separation in reaction centers and description of electron transfer chain, formation of ATP and Calvin cycle. At the end of the Introduction there is more detailed description of photosynthetic antennae which is related to the main topic of the thesis. In summary, the Introduction is sufficiently informative although I found few factual inaccuracies and also organization of the sections could be better. Namely, eukaryotic phototrophs contain also other pigments than chlorophylls and carotenoids (page 1, end of the first paragraph), for instance red algae and Cryptophyta also contain phycobilins. In the description of electron transfer chain (page 5) parts of the text are not fully balanced, there is detailed description of photosystem II (PSII) but information about photosystem I (PSI) and and cytochrome b6f complexes is much briefer. Was there any reason for more detailed description of PSII, otherwise it would be better either to remove some details about PSII or add more details about PSI and cytochrome b6f complex. Finally, the sections 1.2.2. and 1.3. in the last part of the Introduction should be merged since both describe antennae of Heterokontophyta members Nannochloropsis and Trachydiscus.

The following chapter (Material and Methods) describes starting material and basic methodologies used in the work. Mostly I consider it sufficiently descriptive although I miss better explanation of method (and program) used for fitting fluorescence spectra. Clear native electrophoresis instead of clean native should be used throughout the text.

The Results section describes analyses of separated centrifugation zones, gel fractions and chromatographic fractions by spectroscopic methods and by gel electrophoresis. The first part deals with the choice of proper detergent for thylakoids solubilization and then the solubilized pigment-proteins were separated by sucrose gradient. Obtained zones were further fractionated by native gel electrophoresis and or ion exchange chromatography. Chromatic adaptation was finally studied by comparison of zone 3b and especially zone 4 enriched in red pigments isolated from cells acclimated to different light conditions. Due to a complex nature of analyzed samples the results are sometimes rather uneasy to follow, in some case author could make it easier to the readers, for instance by the same orientation of each gel in figures with a pair of gels or by use of the same color for the fluorescence spectra in Figs. 30 and the same ones used for fitting the experimental spectra in Fig. 33.

In the discussion section, author first justified choice of alpha-dodecyl maltoside for solubilization. In the case of digitonin the comparison suffers from too low concentration (0.02%) of the detergent in the gradient which does not reach critical micellar concentration for digitonin. Therefore, the larger complexes aggregated and were precipitated at the bottom of the tube which is well visible (Fig. 15). This obviously disturbed the separation of zones. Author discusses also the chlorophyll-protein composition of the thylakoids and finally the mechanism for chromatic adaptation, especially the red shift in absorption. I found interesting a good agreement between complex alignments based on their size on one side and fluorescence maxima on the other side.

Formally the thesis has a classical IMRAD organization and is written in English which sometimes suffers a bit from improper choice of words and phrases. Also the text flow could be better, on the other hand I found almost no typing errors.

Other questions:

1. On page 5 author mentioned that "The final composition of PSII is species dependent. However the dimeric core which is made of four polypeptides (39 kDa PSII-A/D, 56 kDa PSII-B and 51 kDa PSII-C) and the oxygen evolving complex are preserved (Vinyard *et al*. 2013)." So, which components of PSII are species-dependent?
2. On page 9, author mentioned that photosynthetic organisms contain so called antennae systems "to regulate the energy available for reaction centers" but in the following sentence says that "The main role of these antenna complexes is to provide more energy for photosynthetic reaction centers". What is true?
3. On page 10, author proposed for location of PSI antennae the following reason: „The reason why the antennas are tightly bound only on one side may be the ability of PSI to form trimers in photosynthetic cyanobacteria, from which eukaryotic photosynthetic apparatus evolved. Photosystem I in the potential PSI trimer would then be accessible for integral antennas only from the side, which is occupied on figure 9." So, does plant PSI make trimers?
4. Were the analyses shown in Results performed once or more than once and if more, were they well reproducible?
5. Figs. 16, 17, 21 and 24, were proteins designated PSI-A/B, PSII-A,B,C,D and LHC proteins identified just by analogy with report Grouneva et al?
6. Is it possible to identify the proteins in the gel by mass spectrometry (in other words, are the protein sequences publicly available for Trachydiscus?)
7. How do you explain no red fluorescence in zone 2 which has the highest amount of LHC-R, is it possible that the protein designated as LHC-R in the zone 2 is not LHC-R ?

In conclusion, the thesis showed that author proved to be able to successfully handle various biochemical methods and using them he obtained interesting results. Despite several small problematic issues mentioned above I recommend this thesis for the defense with proposed evaluation very good.

Josef Komenda

In Třeboň, April 22, 2015 ..... Josef Komenda

Review of the master thesis „Light harvesting complexes and chromatic adaptation of Eustigmatophyte alga *Trachydiscus minutus*" by Bc. Marek Pazderník

The submitted thesis by M. Pazderník is an extended and improved version of the original work. The thesis deals with two problems: a) optimization of the method for separation of light-harvesting complexes from the algae *Trachydiscus minutus* using sucrose gradient and b) elucidation of the red shift in absorption and room temperature fluorescence emission spectra of cells grown under red light. For this study author had to learn a variety of biochemical and spectroscopic methods (isolation of thylakoid membranes, sucrose gradient separation, SDS-PAGE, native electrophoresis, ion exchange chromatography, measurements of absorption and fluorescence spectra).

The great part of the chapter *Introduction* contains a general introduction about the structure and function of the photosynthetic apparatus and it has rather a textbook character. As the thesis is focused on the study of chromatic adaptation, I miss more published findings about the chromatic adaptation responses in algae.

The used methods are clearly described in the chapter *Materials and methods*, together with the short explanation of the principles of used methods. I have one question concerning the measurement of light intensity. What is the spectral range of the measured intensity (15 μmol m^{-2} s^{-1}) of growth lights with different quality (low light and red light conditions)? As the spectrum of red light shows a significant contribution above 700 nm (Fig. 11, p. 14), it is important because Hansatech sensors measure PAR region, not the whole region of used red light source.

In chapters *Results* and *Discussion* the obtained results are presented in 29 figures, documenting a great amount of experimental work. The description of results is clear and understandable, results are properly discussed. In Figs. 12 and 13 (p. 19), the spectra for HL and RL cells are compared. What is situation in cells grown under low light conditions? Why spectra for LL cells are not presented, if comparison of responses is made between RL and LL-grown cells (chapter 3.2)? In p. 28 (last paragraph of the chapter 3.1.3) it is not explained why for the investigation of RL-grown cells the concentration of α-D-maltoside was incresed to 3%, why the concentration was not the same (i.e. 2,5%) as in the experiments aimed at optimization of sucrose gradient separation.

The number of references is sufficient, I appreciate that most of them are recent. Four references in the list (Calvin 1989, Kügler et al. 1997, Schmid et al. 1997, Wientjes et al. 2009) are not cited in the thesis. I have another comment of formal nature: The form of the references is not the same. The names of some journals can be found both in the full and abbreviated form (Biochim. Biophys. Acta – Biochimica et Biophysica Acta; J. Phycol. – Journal of Phycology, Photosynth. Res. – Photosynthesis Research).

Overall, my evaluation of the master thesis by Bc. Marek Pazderník is very positive. The comments mentioned above are only of formal nature. The thesis contains interesting findings, it is written clearly, English is at a good level, goals are clearly specified and fulfilled.

In Ostrava, 21st April 2015

Mgr. Michal Štroch, Ph.D.
Department of Physics, Faculty of Science, University of Ostrava