Review of USB FFPW PhD thesis

First name(s), surname, titles of the PhD student: MSc. Anushree BACHHAR	**First name(s), surname, titles of supervisor:** Mgr. Jiří Jablonský, Ph.D.
Title of PhD thesis: Role of isoenzymes in metabolic regulation of cyanobacteria	

REVIEWER:

Surname: Hagemann	**Institution**: University of Rostock, Faculty of Mathematics and Natural Sciences, Institute of Biosciences, Department of Plant Physiology
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Please describe your professional relationship to the PhD student: MSc. Anushree BACHHAR visited my laboratory for several month in 2022	**Please describe your field of expertise:** Cyanobacterial physiology and molecular biology

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field **(extent ¼ – ½ page)**:

Ms. Bachhar's doctoral thesis deals with an important topic – The role of isoenzymes in metabolic regulation of cyanobacteria. Cyanobacteria are an ancient group of prokaryotes, who invented oxygenic photosynthesis. During evolutionary times, cyanobacterial strains adapted to many diverse environments. This flexibility became possible due to the highly versatile metabolism. Among prokaryotes, cyanobacteria possess a very high number of pathways in the primary carbon and nitrogen metabolism, which are highly interacting with each other. In the last decades, cyanobacteria became also an interesting object for biotechnology, since they are regarded as green cell factories capable of producing bioenergy and feedstock from sun and CO_2. However, the obtained productivities are comparable low and need to be improved during the near future.

Hence, understanding of primary metabolism is important to study environmental adaptation's and improve the applied research. For this purpose, new methods are necessary. In addition to lab work on single enzymes or single pathways, a comprehensive overview on metabolism can be obtained by different modeling approaches. Genome models of the cyanobacterium Synechocystis have been published about 10 years ago. In the present study,

kinetic modeling is used, which has advantages in the describing and predicting metabolic fluxes. Specifically, this approach permits the analysis of specific roles of isoenzymes in metabolic networks on a quantitative level. The here used approach is innovative and allowed for the first time to analyze two key questions: First, to which extent contribute phosphoketolase isoenzymes to the growth of cyanobacteria under specific conditions. Second, which role play specific glycolytic pathways, especially the ED pathway in the metabolism of cyanobacteria. This two studies represent the backbone of the thesis and were both published recently in peer-reviewed international journals. These publications are state of the art and allow many new and interesting insights into the regulation of metabolic fluxes in cyanobacteria. They will have a great impact on future genetic engineering strategies.

Elaboration of the PhD thesis, objectives of the work and deliverables

Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicality of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis **(extent ¼ – ½ page)**:

Ms. Bahhar's doctoral thesis aimed to understand the role of isoenzymes in metabolic regulation of cyanobacteria. The thesis is structured accordingly. She starts with a short introduction into cyanobacteria, cyanobacterial metabolism and the general role of isoenzymes. Then, she is defining a list of isoenzymes involved in primary carbon metabolism. This nearly complete list (see my remarks below) is then evaluated in more details. In many chapters' phylogenetic trees permit an overview, whether or not these isoenzymes are widespread in the cyanobacterial radiation or if they may origin from horizontal gene transfer events. The role of isoenzymes is then analyzed by in silico modeling. The kinetic approach is well suited for this purpose. Two case studies on phosphoketolases and the ED pathways were already published. Both publications represent surprises. For example, the different phosphoketolase isoenzymes may be specifically linked to interacting pathways under specific conditions and their modulation will have distinct impact on cyanobacterial growth and productivity. For the ED pathway, the candidate calculated almost no flux through this specific glycolytic route. Interestingly, the candidate proposed an alternative role for the ED enzymes in the glutamate metabolism linked to the TCA cycle and proline biosynthesis. These results are highly interesting, however, they await verification by corresponding strain engineering in the future. Finally, the results are summarized in a general discussion. Here, the model approach is to some extent described and evaluated, which should be improved to give also the non-expert reader more insights in this modern, newly arising field of metabolic modeling.

OVERALL COMMENTARY ON THE PhD THESIS

Please write in the box specific comments concerning the PhD thesis in extent of 1-2 pages:

The thesis is generally well written. Unfortunately I found some cases that need correction and better explanations. The most important things are outlined below:

Page 3; „*Synechocystis* is one of those cyanobacteria that have highest number of isoenzymes coding genes in their genome“ comment: This statement certainly depends on the genome size. There are many cyanobacterial strains having two to threefold more genes than Synechocystis and it can be assumed that they contain more isoenzymes as well. Hence, this statement is only true when comparing Synechocystis with another model strain Synechococcus elongatus having a smaller genome than Synechocystis.

Table 1.1: some important enzymes of primary C metabolism are missing, e.g., phospho-glucomutases, and enzymes involved in glycogen metabolism, which are also belonging to the central carbon metabolism.

Page 5: OXIDATIVE pentose phosphate pathway

Figure 1.1: quality of the figure is not good; it should be replaced by a figure with better resolution!

Figure 1.2: may be previously mentioned and especially studied isoenzymes could be somehow marked?

Figure 1.2: I can not find the glycogen sink, which is mentioned in the legend to Fig. 1.2.

Page 8: please correct text and formula, SBPase converts SBP into DHAP and E4P!

Chapter 1.3.3: this reaction is not reversible, the phosphatase just removes one Pi from the sugar-bisphosphate and the re-addition of P needs an ATP-dependent kinase.

Page 11 and elsewhere in text: what means light heterotrophic? I think that is mixotrophic, i.e. light and glucose, while only glucose and dark is heterotrophic, and only light is autotrophic. The candidate should define the three growth modes of Synechocystis in the introduction and then use always this terminology!

Chapter 1.3.4. there are no higher and lower organisms in biology, probably a better word would be in eukaryotes

Page 12, PFK A2 in Bacillus: be careful, this enzyme of 100% identity is in a Bacillus strain expressing all the genes from Synechocystis, hence it is the PFKA2 from 6803!

Chapter 1.3.6 - if the reaction is reversible, please indicate it by two arrows in both directions

Page 15: acety production? I think you mean acetyl-phosphate! I think most acetyl-phosphate is made from pyruvate due to PDH reaction which might be mentioned. By the way, PDH has also two isoenzymes in Synechocystis analyzed by Wang et al. 2022.

Chapter 1.3.7: PGA mutases are directly and reversibly converting 2PGA into 3PGA and vice versa, there may be an enzyme bound intermediate in the reaction cycle, 1,3bisPGA. More importantly this metabolite has regulatory function to one isoenzyme, but it is not released, please correct the formula and include the metabolic regulation as well.

End of chapter 1.3.7: please include the paper by Orthwein et al. 2021, which verifies the enzyme activity and regulation of the main PGA mutase isoenzyme in Synechocystis

Chapter 1.5: Regulation of isoenezymes is an important issue to understand the role. The candidate discusses some aspects of the transcriptional regulation. In this regard, the candidate may refer to the data base CyanoExpress (http://cyanoexpress.sysbiolab.eu/), which summarizes known transcriptomic data for each gene in Synechocystis 6803. More importantly, regulation on protein level by protein phosphorylation and/or redox level might be much mor important to regulate the flux through different isoenzymes. This layer of regulation is not mentioned and needs to be explored, at least with some examples.

Chapter 4.1.- general discussion: I would like to see a better explanation of the model approach. The candidate should compare it to other model approaches in the filed and should give a real discussion about the strength and weakness of the used modeling compared to others. This point should be also discussed in the thesis defence.

In general, I enjoyed reading the thesis. All in all, Ms. Bachhar has clearly shown in the present work that she is capable of largely independently working on a scientific problem. I recommend the Faculty accept this promotion.

FINAL RECOMMENDATION

☐ **PhD thesis can be recommended for defence**
X PhD thesis can be recommended for defence with reservations
☐ **PhD thesis cannot be recommended for defence**

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20.12.2022, Rostock

Prof. Martin Hagemann

Date and place

Name and signature