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Posudek disertační práce/Opponent PhD Thesis Review:

Ivan N. Ivanov: Coordination of Growth and Cell Cycle Progression in Green Algae

The PhD thesis of Ivan Ivanov focuses on cell cycle and growth dynamics in two representatives of green-lineage algae, namely *Desmodesmus quadricauda* and *Chlamydomonas reinhardtii*. It explores of the effect of temperature on the cell cycle dynamics in *D. quadricauda* and supra-optimal temperature on the cell cycle arrest in *C. reinhardtii*. Following the finding of elevated starch accumulation upon cell cycle arrest under supra-optimal temperatures, further work aims at establishing the potential use of the cultivation strategy in biotechnological scale. The last part of the thesis is more methodical, establishing Raman microscopy for the quantification and distribution of storage compounds in the cells of *D. quadricauda*.

The thesis is written in English and is separated into 8 chapters, where chapters I and II are introductory and present the goals and hypotheses, and chapter VIII is summarizing. Chapters III – VII compile 3 published articles (*Journal of Experimental Botany*, *Cells* and *Folia Microbiologica*) and 2 manuscripts. In addition, Ivan Ivanov is a co-author of another article (*Algal Research*) published during the PhD study that is not featured in the thesis.
Ivan Ivanov is the first author of one review article in *Folia Microbiologica* (Chapter III) and one manuscript (Chapter VI) and second author of 2 published articles (Chapter IV, V) - and one manuscript (Chapter VII).

I general, I find the thesis well structured, easy to read, with minimum formal and language issues. This applies also to parts that have not undergone peer review, namely the introduction, manuscripts and summary. The content makes a very interesting read and the chapters are well assembled into a logical and consistent piece of work. I however found the text of some parts of Chapter I to be almost identical with the article published in *Folia Microbiologica* (Chapter III) – (e.g. see pg. 8 and pg. 51 left column). Although this is undoubtedly connected to the strategy to publish the thesis introduction and the candidate is the first among three authors of the published article, I think original text should have been produced to document the author´s sole contribution.

What I would have also appreciated in the thesis is:
1. Clear statements of the candidate´s particular contribution to each article or manuscript.
2. More detailed introduction in terms of information content and figures. I think it would be good to provide theoretical background to the methods used by the candidate himself, including theoretical description of strategies used to determine growth parameters of cultures, kinase activities or storage compound determination. This information can be found in pieces throughout the articles and manuscripts but given that all these works are collaborative, the

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experimental approaches mastered by the candidate are unclear. In addition, a figure summarizing the current state of knowledge on the described molecular mechanisms governing cell cycle progression would have been helpful.

3. More elaborate and detailed discussion in Chapter VIII putting the novel findings provided by the thesis into the context of available literature, current knowledge and state-of-the-art.

Based on the comments above, I have the following questions for the candidate:

1. Can you please summarize methods that you were yourself carrying out and detail the procedure for determination of CP establishment and kinase activity assay?
2. Based on available data and your results, especially those showing the different effect of light and temperature in the relationship between CP and cell size (see summary pgs. 164, 165), can you suggest a current working model for the determination of final daughter cell number in the multiple fission cell division cycle? Considering there is (or should be) a critical size threshold of the daughter cell, do you think the number of daughter cells rather pre-determined by the mother cell (size/metabolic state etc.) or is it determined by the final (minimal) size of the daughter cells?
3. Polyploid cells are frequently found in metabolically active tissues (e.g. mammalian liver or placenta, insect salivary glands or plant leaf mesophyll). In your supra-optimal temperature cultivation system, are there indications that ploidy level may be one of the factors contributing to the capacity of a cell to produce/accumulate storage compounds? Can ploidy level of a cell limit its metabolic potential and may targeted manipulation of cell cycle to increase ploidy level by itself contribute to storage compound production? If so, can you suggest potential strategies considering the multiple fission cell cycle?
4. Based on available knowledge, your results and experience, what are your recommendations (e.g. to a company) for the best strategy to enhance starch production in "green" microalgae? Do you think similar principles can apply to other species? Is it expected that other species would also be similarly prone to biofilm formation, which was limiting the light availability and productivity?

Despite some critical points, I think that the PhD thesis fulfils the requirements for successful defence and look forward to the defence and discussion.

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Opinion on the PhD dissertation

Ivanov, I.N. (2020): Coordination of growth and cell cycle progression in green algae. - PhD thesis, Univ. South Bohemia, Č.Budějovice, No. 17, 172pp.

The work is based on 5 publications, three have already been published, here is the candidate one of the co-authors, in two of them is the first author. At the same time, it critically evaluates a large number of publications of world literature in the field.

Topic: is very well selected, the growth and control of the life cycle of cells, in general, is a fundamental topic of basic research and has logical connections to biotechnological applications. Even with algae, this is the topic of many publications, as evidenced by an extensive list of studied literature. It is nice that the author also cites older works, even Annual Reports!

Methods: During his work, the candidate mastered classical as well as modern methodologies of life cycle research and algae physiology, incl. promising Raman cell spectra.

The goals of the work are clearly defined. I would just like to remind you that the focus on the production of starch by algae for biotechnology is somewhat unpromising, sugars and starch are without problems and economically produced by conventional agricultural crops. However, polyunsaturated fatty acids are irreplaceable, they are a specialty of algae, as well as special substances such as carotenoids, astaxanthin, fucoxanthin, ECP, etc. biologically active substances, as the author correctly discusses. I would recommend concentrating on them in the next work. However, the relationship between the production of starch and subsequently oils demonstrates the importance of studying the conditions and possibilities of regulating algal metabolism in general.
The author rightly highlights the application possibilities of algae metabolism regulation by nutrients starvation, changing the temperature and light conditions, and the multi-stage cultivation methods. These are currently the methodologies most widely used in biotechnology.

Selection of model organisms: The author extracted a huge amount of knowledge from the workplace obtained on the alga *Desmodesmus* (*Scenedesmus*) *quadricauda*, *Chlamydomonas reinhardtii* and *Chlorella* sp. and *Parachlorella* (*Chlorella*) *kessleri*, all traditional model organisms in Třeboň laboratory. In the literature review, however, he also discusses models from other groups of organisms. The candidate made good use of the knowledge gained in the workplace with the regulation of the algal cell cycle and incorporated it into his experiments.

Results: The most important is probably the finding that the critical volume of cells is not the main trigger mechanism of cell division, but that it is the activity of cyclin-dependent kinase. Furthermore, that temperature mainly controls growth rate and metabolism. Supra-optimal temperature produces large cells with a higher starch content, which is useful for

biotechnology. The fundamental importance of light intensity, i.e. the supply of energy to cells, is also shown.
Considerations of the possible use of supra-optimal temperature to stimulate starch production in biotechnology are interesting. Large-scale cultivations in an un-tempered greenhouse during cold period of year are a welcome possible extension of the cultivation period, but they will encounter a lack of light. In the polar regions, however, it would be possible, there the light is weaker, but constant, so that the overall energy input is even higher than in the tropics (12 h light and 12 h dark).

I also do not see the perspective of the production of bioethanol as a fuel by fermentation of starch from algal culture. Starch must be separated, broken down into sugars, fermented and ethanol distilled, which means a number of losses and costs. In addition, the production of sugar from cane and starch from corn or potatoes is and will probably be more economical for a long time (which the author also correctly states).

Comments: Algal biotechnology is older than since Sphoer (1953). Harder et Witsch (1942) published a study on the use of diatoms to produce oils as lubricants for the military. Them cites Řetovský, R. (1946): Mass cultures of some unicellular algae. - Studia Botanica Čechoslovaca 7 (1): 38-48. Řetovský successfully operated the algae culture *Scenedesmus obliquus* and *Navicula* sp. in a volume of 70 L! Diatoms were grown to produce fucoxanthin. He also illuminated his "megalocultures" with artificial light. At the time, both of these studies were pioneering.

In chap. VI. I miss a bit of measuring the light input from the Sun to the culture vessels, even though it was the source of most of the energy.

A list of abbreviations for the whole work, in one place, would also be a relief for readers.

Formal arrangement: The work is written in an exemplary manner, it can be recommended as an introductory study text for those interested, it is very nicely written.

Conclusion: The submitted dissertation meets the requirements for the award of a PhD degree very well, so I can gladly **recommend it for admission and a candidate for the award of a PhD degree.**

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