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Review – PhD thesis of Mgr. Eva Hejduková "Freezing tolerance of freshwater diatoms as a key to their success in polar regions"

Polar diatoms are confronted with pronounced seasonal variations of solar radiation and low temperatures conditions in summer, as well as long periods of ice and snow cover during winter. Many freshwater habitats are only ephemeral, and hence the inhabiting limnic diatoms are regularly exposed to desiccation and freezing stress conditions. There are manifold protective mechanisms described in polar microorganisms from the molecular level up to life strategy changes such as switching to dormancy or the formation of resting stages, but for polar freshwater diatoms many gaps in fundamental knowledge still exists.

Therefore, in the present PhD thesis **Eva Hejduková** investigated various freshwater diatoms isolated from Antarctica and the Arctic using field and laboratory based approaches. On Svalbard seasonal changes of freshwater diatom communities were studied using monitored environmental data along with a set of fluorescent stains which were used as cell biological tools to visualize and quantify various viability markers such as membrane integrity. Here in particular the winter data are of great scientific value. In the lab experiments **Eva Hejduková** used clonal diatom cultures isolated from the polar regions to evaluate their freezing tolerance under controlled temperature gradients. The data indicate that polar freshwater diatoms are able to survive mild freezing (−4 °C) with high viability, while they did not tolerate lower temperatures (−20 °C) as reflected in enhanced mortality. Interesting is the observation of resting cells which exhibited a higher freezing tolerance compared to vegetative cells. All these data contribute to a better understanding of the ecophysiology of freshwater diatoms from Antarctica and the Arctic.

The cumulative PhD thesis of **Eva Hejduková** consists of a general introduction and synthesis followed by 4 publications, 3 with first authorship and 1 with co-authorship. Three papers are published in internationally acknowledged, peer-reviewed journals, one as a book chapter.

Therefore, I like to highlight that **Eva Hejduková** has a very good publication record in terms of her career status.

The PhD thesis is generally well structured as it contains an introduction into the diatoms, polar environmental conditions and protective mechanisms of microalgae against abiotic stressors. This introduction equals in many aspects a synthesis and is followed by the research objectives and a summary. Most of the aspects are comprehensively discussed in the frame of about 300 references.

Nevertheless, I have also to mention some critical points:

1. I miss at least a small chapter on diatom origin and evolution, i.e. on their chimeric genome with contributions from very different organisms. This genetic "mixture" led to unique combinations of metabolic pathways (animal- and plant-like lipid metabolism etc.) that contribute to the high physiological plasticity, often wide tolerances and ecological success of this relatively young algal group (compared to Chlorophyta and Cyanobacteria).
2. It is not always clear which statement in the introduction is directly related to freshwater diatoms, other diatoms or even other algal groups. The candidate should be aware that algae represent phylogenetically different lineages derived from primary, secondary or even tertiary symbiosis which reflects different molecular, biochemical and physiological traits. I would have preferred a better structured text, each chapter always starting with all facts on freshwater and marine diatoms followed by research data on other algal groups.
3. I am also not happy with some citations, i.e. how the information from the paper has been used. To give one example – Arnold et al. (2003) studied carbohydrate patterns as source for protective compounds from soils on Signy Island. Although the candidate mentioned in her thesis soil microalgae as source for the measured compounds (Table 2), a closer look on the original data clearly indicates lichen-dominated cryptogamic communities growing on the soils. This makes a big difference in the arguments and conclusions since the lichen photobiont and mycobiont as well as associated moss, microalgae, cyanobacteria and heterotrophic bacteria could be a source for the mentioned compounds. As a consequence the discussion should be much more differentiated.
4. In addition, some of the analytical methods in Scholz & Liebezeit (2013) are questionable for unambiguous identification of protective compounds. Photometric assays or thin layer chromatography have many drawbacks, and hence HPLC or GC coupled MS techniques are required for proper chemical identification/screening of osmolytes/compatible solutes. A better reflection of the literature is desirable.
5. The cryoprotective function of DMSP has been experimentally proven with enzyme assays (Karsten et al. 1996, Dimethylsulfoniopropionate production in phototrophic organisms and its physiological function as cryoprotectant. Biological and Environmental Chemistry of DMSP and Related Sulfonium Compounds, R.P. Kiene, P.T. Visscher, M.D. Keller and G.O. Kirst (eds.), Plenum Press, New York, 143-153; Nishiguchi & Somero 1992, Cryobiol.)
6. Freezing of algal cells is a complicated process. If cells loose water this automatically increases the osmotic potential inside, i.e. all concentrations of inorganic ions and organic compounds rise, which in parallel decreases the freezing point of the cytoplasm/vacuole. Because of this physico-chemical process - in which no metabolic energy is involved – many polar algae can cope relatively well with frost and freezing. On top of this process many algae synthesize/accumulate additional osmolytes for further lowering of the freezing point and/or small proteins to influence extracellular ice formation.

7. From a biochemical standpoint microalgae (and other biota) can follow a qualitative, quantitative or modulation strategy to optimize their enzyme activity under low temperature.
8. How is diatom migration considered/assessed compared to biochemical photoprotection?
9. What means "internal valve formation"?
10. There are many putative osmolytes/anti-freezing substances mentioned in the thesis – is the postulated function really experimentally proven if you consider molecular size and structure as well as physico-chemical properties for the most important compounds?
11. I miss an outlook

In summary, in spite of my critical points, I think the present PhD thesis of **Eva Hejduková** represents an interesting piece of original research, fully comparable to the international standards, and hence should be accepted without any problems by the Faculty of Science, Charles University in Prague.

My recommendation: **pass**

(Prof. Dr. Ulf Karsten)