Alena Štrojsová: Expression of extracellular phosphatases in phytoplankton populations at the single-cell level (České Budějovice 2005, 105 p.).
A review of the PhD. thesis

The thesis consists of an extended abstract, three published manuscripts and three manuscripts submitted for publication. The applicant is the first author of all these manuscripts but one.

The first paper (2003, Eur. Journ. Phycol., 38: 295-306) is a seasonal study of extracellular phosphatase expression in phytoplankton of the eutrophic reservoir Římov. The fundamental pre-requisite of this study was a modification of the existing method, which made possible successful measurements of extracellular phosphatase activity in both single cells and in the whole phytoplankton. The study revealed different activities during the season, in the major taxonomic groups and in individual species as well. Some species never expressed any activity at all.

The second paper (2005, Eur. Journ. Phycol., 40:251-258) is a similar study carried out in a highly eutrophic, subtropical and polymictic lake in China. The study period was confined to April and May. The results were in general accordance with the previous study, but the relationships between bulk phosphatase activity and either SRP and chlorophyll *a* were very weak. This could have been brought about (at least partially) by an unexpected high phosphatase activity in heterotrophic nanoflagellates.

The third paper (2005, Marine and Freshwater Research, 56:417-424) brings evidence that several phytoplankton species regulated phosphatase activity according to the external phosphorus concentrations; other species did not produce extracellular phosphatases at all.

The first manuscript (submitted to Environmental Microbiology) is a study on diurnal changes in extracellular PA in the eutrophic Římov reservoir, in the acidic lake Plešné and in a batch culture of *Chlamydomonas reinhardti*. The PA varied greatly in time, but the pattern was rather unpredictable.

The second manuscript (submitted to Environmental Microbiology) poses a question whether the production of extracellular phosphatase is advantageous in the competition of phytoplankton species. The output of the experiments seems to indicate that the production of EP is only one of many strategies of survival in phytoplankton.

The third manuscript (submitted to Polish Journal of Ecology) is a review of the use of ELF (Enzyme Labelled Fluorescence) technique in phytoplankton studies.

Reviewer's comments to life strategies of phytoplankters which do not show any significant phosphatase activity at their cell surface.

(i) Most flagellates (Chrysophyceae, Dinopyceae, Euglenophyceae, Cryptophyceae, Chlamydophyceae) were scarcely or never phosphatase – active. One reason may be the mixotrophic (or even phagotrophic – namely in *Dinobryon*) capabilities of the flagellates, as mentioned on the page 13 (extended abstract of the thesis).

The other reason might be their motility. An actively swimming cell (contrary to a non-motile cell entrained in turbulent eddies) breaks down the concentration gradient that may develop at the cell surface and the moving cell may encounter small patches of increased phosphate concentrations. All these mechanisms may be substitutes for providing phosphate by hydrolysis at the cell surface.

(ii) Large phytoplankters, like *Staurastrum planktonicum*, but also some large diatoms, are not susceptible to zooplankton predation. When living in a well mixed column they can survive without

maximizing their growth rates, so that a fast uptake of phosphate is not necessary. This may hold also for large, non-dividing cells of *Planktosphaeria gelatinosa* which are common at the spring clear-water phase (did you ever find any phosphatase activity at the surface of these large cells?)

Conclusion

From the papers submitted as the present thesis it follows that the author brought a significant contribution to the knowledge of ecology of freshwater phytoplankton. I consider that the thesis can be validly presented by Alena Štrojsová to obtain the degree of doctor of philosophy (PhD) at the South Bohemian University.

Prague, 10th January 2006

RNDr. Jan Fott CSc.

Evaluation of the Ph.D. thesis of Alenka Strojsova
'Expression of extracellular phosphatases in phytoplankton populations at the single-cell level'

One of the key issues in phytoplankton production concerns the factors limiting primary production. Commonly, carbon dioxide is not limiting, light availability might limit phytoplankton production in highly turbid waters when the mixed layer is deeper than the depth of light penetration. Most commonly, however, inorganic nutrient availability limits phytoplankton production. In freshwater systems this limiting nutrient is most frequently phosphate. Phosphate is cleaved from organic phosphorus compounds using the hydrolytic enzyme phosphatase, commonly expressed by bacteria and a large number of phytoplankton species. For phytoplankton expressing extracellular phosphatase, this represents a strategy to become more independent from phosphorus regeneration by the heterotrophic community of the microbial food web. This hydrolytic enzyme responsible for the cleavage of phosphate from organic P-compounds in the ambient water is located outside the cell membrane and hence, coined extracellular phosphatase. There are numerous reports on the regulation mechanisms and hydrolytic potential of extracellular phosphatase in microbial plankton communities in the natural environment and on specific microbial strains (bacteria and phytoplankton) in cultures. The question, however, how this phosphatase activity is distributed among different members of individual populations and communities, however, remained enigmatic until recently since there were no single cell approaches available to determine extracellular phosphatase activity of individual cells.

In the present Ph.D. thesis, this problem has been addressed by combining bulk phosphatase activity measurements as previously done with the recently developed single cell approach using ELF-phosphate.

The first chapter of the thesis focuses on seasonal dynamics of the phytoplankton extracellular phosphatase activity in a eutrophic reservoir combining bulk phosphatase activity measurements with a modified version of the ELF-substrate measurements. First the modified ELF-substrate was compared with the bulk measurements, concluding that both methods detect most likely the same group of phosphatases. The use of the ELF substrate allowed obtaining a better insight into the distribution and dynamics of phosphatase activity among the different phytoplankton taxa. Some of the major groups of phytoplankton dominating the phytoplankton community (cryptophytes, chrysophytes) in terms of abundance never or hardly expressed phosphatases. In total, 56 species were identified over the season cycle of which about one third never expressed any phosphatase. Other pgytoplankton groups, such as cyanobacteria and chlorophytes expressed phosphatases only in the summer, while others essentially always express this enzyme.

In the second chapter, the dynamics of phosphatase activities in a eutrophic, highly turbid lake are studied. In contrast to the findings described in the first chapter, the cyanobacterial community of this lake never expressed phosphatase activity. Some of the phytoplankton species exhibited phosphatase activity even when ambient phosphate concentrations were high and evidence is presented that heterotrophic nanoflagellates also contributed substantially to the pool of extracellular phosphatase.

The regulation of extracellular phosphatase activity under contrasting ambient phosphate concentrations, i.e., endproduct regulation of phosphatase, is studied in the third chapter. Here transplantation experiments were performed, transferring phytoplankton from phosphorus-replete conditions to a P-limited environment and vice versa. While cyanobacteria and some green algae did not express phosphatase activity at all, thus confirming the results of the first chapter performed at the same study site, a number of phytoplankton species (diatoms and some green algae) regulated their extracellular phosphatase activity according to the ambient phosphate concentration, thus, via the concentration of the endproduct.

In chapter 4, diurnal changes in the extracellular phosphatase activity of natural phytoplankton assemblages are shown. Additional experiments on this subject were performed in the laboratory using batch cultures of *Chlamydomonas reinhardtii*. Although there were considerable dynamics in phosphatase activity recorded over diel cycles, the pattern in phosphatase activity seems not to be governed by day-night cycles. It is concluded that there are other factors controlling phosphatase activity in lakes potentially masking diel patterns such as mixing of the water column but also changes in the microbial community and differential responses among different compartments of the microbial food web.

The fifth chapter investigates a central question in ecology, i.e, whether a specific ability or feature in a specific species offer a selective advantage over other, similar species. The expression of extracellular enzymes should have some energetic costs, which might lead to a lower growth rate than competitors which do not express this enzyme. In this study, first the expression pattern of phosphatase was determined under P-replete and P-deplete conditions. It was found that under P-replete conditions less phytoplankton cells are expressing phosphatase. Among those expressing phosphatase under P-replete and deplete conditions, those cells growing under P-replete conditions exhibited higher growth rates. Thus, it is concluded that the expression of phosphatase helps to survive P-limited conditions but does not increase the competitiveness against phytoplankton species not expressing the enzyme if sufficient P is available. One might expand this study and try to quantify the energetic costs of expressing phosphatase. As far as know, this has not been done thus far.

In the final chapter 6 the potential of the ELF technique is reviewed to assess single cell extracellular phosphatase activity.

Overall, the presented Ph.D. thesis, composed of individual papers of which three are published in leading international journals, two others are submitted and one review paper is submitted, is of very good international standard. The candidate developed a recently introduced method further and modified it allowing its use in ecological studies and used this modified method in different ecosystems combining field measurements with laboratory experiments. Based on the presented thesis, the candidate certainly can be admitted to defend her Ph.D. thesis.

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Review

of the Ph.D. thesis by Alena Štrosová: Expression of extracellular phosphatases in phytoplankton populations at the single-cell level. Hydrobiological Institute, AS CR, 105 pp.

Introduction

Submitted Ph. D. thesis is a part of more complex research devoted to study of nutrient limited, mainly under P-deficiency, planktonic community of both lakes and reservoirs which is carried out at the Institute of Hydrobiology in České Budějovice at present. A study of extracellular enzymes in water environment has long-term tradition at this Institute and the team under supervision of Dr. Jaroslav Vrba published very interesting results of their research in reputable international scientific journals and these papers are often cited. Among others, Jaroslav Vrba and his colleagues were one of the first teams in the world who introduced use of the substrate ELF97phosphate for routine analysis of P-limited plankton community. So, this study of Alena Štrojsová based on the above mentioned research brings new findings that have enabled to test new hypotheses and assumptions.

Two main objectives of the study could be recognized. The first was optimalization of the ELF technique for use in natural phytoplankton samples and the second one was routine analysis and comparison of phosphatase activity among freshwater and cyanobacterial populations leading to evaluation of the importance of phosphatase expression for species competition.

General evaluation of the thesis

In general introduction, the author shortly summarized up to date knowledge on mutual relationships between phosphorus deficiency in phytoplankton and displaying extracellular phosphatase activity to acquire phosphorus (namely inorganic orthophosphate - P_i) from organic compounds. She defines scope and main aims of her study and outlines research design and approaches involved in the study.

Since the thesis is based on several published or submitted papers, the author included summary of the most important results and findings which contribute to much better understanding and reading of the text.

Regardless of very interesting data presented in the submitted thesis, from my point of view, the most important knowledge resulted from her work is, briefly said, that extracellular phosphatases are probably important for the persistence of particular phytoplankton species in P-deficient aquatic environment, but they cannot cause significant increase in population abundance.

Results of Alena Štrojsová's work are original and bring very interesting and important knowledge that extend our information about dynamic and diversity of phytoplankton assemblages. Of six presented papers or manuscripts, three of them were already published in the reviewed journals with impact factor larger than 0.5, three remaining were submitted to international journals. Alena Štrojsová is the first author of the 5 papers. Thus, based on published papers, I am sure that results presented in this thesis, should be of great value for other scientists and no other comments or improvements are necessary.

I would only like to put two questions which might be inspiring to the author for her next investigations as well as interesting for explanation to all colleagues during a defence of this thesis:

1) It is supposed that various sources of extracellular enzymes exist in the water (e.g. Vrba et al. 2004). Undoubtedly, this is also a case of extracellular phosphatases (EP) released by different taxonomical groups into the water environment. Is it possible to distinguish between those EP produced by phytoplankton and EP produced by bacterioplankton? According to my knowledge, size fractionation of the water sample is the only tool how to investigate the source of total water enzyme activities.

2) Extracellular phosphatases represent rather survival strategy for subdominant phytoplankton species under conditions of phosphorus deficiency. Can we explain a Hutchinson's paradox of the plankton by this strategy ?

Conclusion

The autor has proved her competence in elaborating and experimental testing of the scientific hypotheses and constructive intepretation of results obtained during her Ph.D. study. I can authorize that submitted thesis meets all requirements for absolvents of the Ph.D. study.

Thus, I highly recommend to accept
Alena Štrojsová's Ph.D. thesis for defence

Olomouc, January 16th 2006

Martin Rulík, Ph.D.