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Review of the doctoral thesis of Hana Medova entitled

Phototrophic microorganisms in extreme environments

The work of Hana Medova deals with the analysis of distribution and physiology of photosynthetic prokaryotes in several extreme environment, such as in extreme saline, thermal, and acidic and polar conditions, etc. The submitted thesis has 123 pages., consists of an introductory chapter, four chapters introducing the method, results and discussion of the main research work, and at the end a summary of the total thesis.

In chapter 2 "*Anoxygenic bacteria in saline and soda lakes*", the authors studied the distribution of anoxygenic phototrophs in more than twenty saline and soda lakes, by using IR epifluorescence microscopy, IR flurometry and pigment analysis. As a result, they found that anoxygenic phototrophs are abundant in shallow, fully oxic lakes, while in highly mineralized lakes there was little APB, likely due to the lack of availble oxygen. And concluded that AAPs may play important roles in the shallow soda lakes. In chapter 3 "Anoxygenic phototrophic bacteria in polar regions", the authours studied the distribution of AAPs in both arctic and antarctic freshwater lakes, and found the exsistence of AAPs in two thirds of the studied lakes. Also, temperature was found to be the most important environmental factor influencing their distribution. In chapter 4 "Thermostatility of anoxygenic reaction centers", the thermal stability of photosynthetic reaction centers in purple phototrophic bacteria was investigated by using infra-red kinetic fluorometry and computer modeling. It was found that purple reaction centers had their forward electron transport optima between 40-75 ℃, approximately 20℃ above their optimum growth temperature. The purple reaction center stability largely depend on tertially structure stabilization through weak interhelical van der Waals interations. In chapter 5 " Effect of post-mining waters on cyanobacterial photosynthesis", the author studied the responses of several planktonic cyanobacteria strains to acidic post-mining waters, and found that acidic pH has more important influences on the cyanobacterial photosystem II, as compared with the high concentration of dissolved ions. Also, the response of cyanobacteria was species-specific and the discharged postmining waters may not prevent the cyanobacterial bloom in newly formed water bodies.

Anoxygenic phototrophic bacteria are an ecologically important functional group of bacteria, which has been extensively studied in the past. Many experiments revealed that this functional group of bacteria are important components of the marine and freshwater microbial world, and play important roles in aquatic carbon cycles. While, so far little is known about their distribution as well as their physiology in the above

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described extreme conditions. The results of this thesis largely improved our understanding of the ecological importance of the anoxygenic phototrophic bacteria, as well as their photosynthetic activities.

The thesis is a high quality work, which fully meets the international standards. A part of the results was already published in high quality international journals. The strong point of the thesis is a robust experimental support elaborated in several detailed studies, which provides a solid scientific base for firm conclusions.

In spite of that I am obliged to raise some comments and questions:

Chapter 2, The authors interpreted that the very low abundance of APB in highly mineralized lakes was likely due to the absence of oxygen in high saline waters. While, in shallow, fully oxic lakes, APB represented as high as 7-65% of total prokaryotes. What's the reason? Is it only because there was enough oxygen in these environment? or are there some other reasons?

Chapter 2, Could the author completely convince that the obtained images by infra-red epifluorescence microscopy are all APB, not concaining purple non-sulfur bacteria or someone else?

Chapter 2, The author found there was a strong positive correlation between the chl *a* and APB abandance, could this also implicate that APB prior to live nearby phytoplankton (or in phycosphere)?

Chapter 3, The authors found that APB existed in two thirds of the studied polar lakes with a temperature higher than 5℃, while in the other one thirds of the studied lakes, some of which had a relatively higher temperature, but APB were absent in these environments, could the author interpret the possible reasons?

Chapter 5, Could the author explain why *microcystis* were more resistant to acid than *Dolichospermum*? Also, as a matter of fact, no cyanobacterial cells could be found in the Lake Medard, would it be too rough to conclude that the actual water chemistry of the upper layers of Lake Medard did not prove to effectively prevent any cyanobacterial bloom?

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The text of the thesis contains some grammatical and spelling errors, which could have been avoided by better proof reading, such as:
Page 1, paragraph 2, line 2, "two fundamentaly different" should be changed to "two fundamentally…"

Page 21, paragraph 1, line 12, "… anoxygenic phototrophs were registred in …" should be revised to "…registered to …"

Page 62, paragraph 2, line 4, "…as it was describe in case of PSII…" , should the word " describe " be deleted?

Page 76, paragraph 1, line 4, "The most variable part of the motif was then its first…", should the word "then" be deleted?

In summary, the work of Hana Medova presents important and new results and proves his ability to conduct productive scientific research. The submitted doctoral thesis meets high international standards. I have no doubt that Hana Medova fully deserves the title *Doctor of Philosophy* Ph.D.

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Review of the Ph.D. thesis of Hana Medová

Title: *Phototrophic microorganisms in extreme environments*

The dissertation of the Ph.D. candidate Hana Medová deals with phototrophic microorganisms from a wide range of extreme environments including extreme salinity, temperature and pH conditions. The submitted thesis has 121 pages and it is well written in English. The thesis is organized in 5 chapters, the first one being the introduction and the other four corresponding to 4 articles, one of which is already published (Chapter 2) and another one submitted (Chapter 5) to high quality international journals (). The candidate is first author in the 4 papers that are the core results of this thesis and in addition is co-author in other two published manuscripts that are relevant to the thesis.

The thesis work is of high quality. The introduction is nicely written and provides a general overview of phototrophic organisms inhabiting various extreme environments as well as basic information on the different mechanisms that microorganisms have to achieve adaptation to these extreme conditions. Since very different aspects of extremophily are approach in this thesis, I find the general introduction very useful and necessary to follow appropriately the other chapters. The candidate did a very good job summarizing the important aspects of the different extreme environments that are relevant to her work.

The core results of the thesis represent multidisciplinary research and include the usage of several state-of-the-art analytical techniques (kinetic fluorometry, epifluorescence and light microscopy, emission spectroscopy, and high performance liquid chromatography). Chapters 2 and 3 provide information on different phototrophic communities inhabiting saline and soda lakes and freshwater polar lakes respectively. Further, Chapter 4 is based on laboratory studies to depict thermal stability of the anoxygenic reaction centers and finally Chapter 5 describes experiments studying the response of planktonic cyanobacteria to acidic post-mining waters. Thus, the work is a nice combination of field and laboratory studies and approached a wide range of topics from basic description of these communities to possible mechanisms of adaptation to extreme conditions. In my opinion, particularly the In particular, the data obtained regarding the Aerobic Anoxygenic Photrotrophs (AAPs) is of great value since despite the advances achieved in the last years in studying this relevant group of bacteria in aquatic systems, most studies have focussed in the

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marine environments or in temperate freshwater lakes. Very little was know up to date about them in extreme environments, thus, this thesis contributes significantly to the field.

In spite of the high quality of the thesis, I have some comments and questions that I must to point out.

In general, the candidate talks about APB (Anoxygenic Phototrophic Bacteria) and includes the AAPs (Aerobic Anoxygenic Photrotrophs) in them. I understand that both organisms can inhabit the studied systems and despite the conditions seem to indicate that the organisms accounted are likely AAPs, Hana Medová uses a more general term since no confirming proof was obtained, but in some points of the thesis this is confusing.

Chapter 2 – This Chapter is in my opinion the best contribution of the thesis, and as proof, it has been published in very good journal (IF=3.4). However, I have some aspects to comment:

In relation to the issue of whether the organisms were in fact AAPs or APBs, is there information of the oxygen concentration of these lakes? I have only seen the information where a depth profile was performed.

The morphotypes observed in these lakes are quite intriguing. Does the candidate or other researchers in the research laboratory have any information about their identity?

Despite the variability in the contribution of AAPs to total prokaryotes in the lakes studied, in general values reported are very high compared to those from marine systems or temperate freshwater lakes. In an attempt to study the bottom-up factors that might influence these abundances, a correlation was found with Chlorophyll a. However, it is well known that AAP are more vulnerable to predation that other bacteria but this issue is not mention in the discussion. Does the student have any information on the abundance of predators in these systems? Does she hasve any indication that a lower presence of predators might be related to these high abundances or at least does she think this can be a plausible explanation?

Chapter 3 – This Chapter presents a survey of bacterial phototrophic community in freshwater polar lakes from both poles. I think this is the weakest chapter of the thesis. Results and discussion are less than 3 pages long, and I think the results need more discussion. Despite being a small survey, the system studied is very interesting and these results provide valuable information of possible the links between AAP and temperature, which is an unclear issue. Surprisingly, this

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chapter in not included in the list of publications relevant to the thesis. What is the status of this manuscript?

Through the analysis of APB, data about picophytoplankton is also obtained, yet is not mention in the chapter. Did you also observe Chl *a*-containing organisms in the majority of the lakes? Did you see any difference in the temperature limit for Chl *a*-containing organisms than for those containing BChl *a*? These results might be interesting to look further if this chapter is prepared as manuscript to be submitted for publication.

Chapter 4 – This Chapter approaches a very interesting and different topic than the others and provides the thesis which a much more multidisciplinary character than normally, which is a merit that needs to be highlighted. What is the status of this manuscript?

Figure 4.2 legend in page 67 refers to the white arrows that indicates the electron transfer. I cannot distinguish this arrows in the image.

The higher optimal temperature of an AAP is that of *Porphyrobacter tepidarius* OT3. It is mentioned in the introduction that this is the higher limit described for an AAP so far. This should have been also noted in the introduction or methods section of Chapter 4.

Results show that cyanobacteria showed lower thermal stability of reaction centers compared to their purple-bacterial counterparts but contrarily, thermophilic cyanobacteria have been described whereas purple bacteria lack truly thermophilic strains. Can you suggest possible explanations?

Chapter 5 – Despite approaching phototrophic organisms in acidic environments, I find this chapter somewhat disconnected from the rest of the thesis. Yet, the research is of high quality and the results very interesting.

My question is that taking into consideration the results of her experiments, which strains would the candidate choose as the most suitable for bioassays of AMD in this region?

Reading this chapter, it is unclear to me why there is a concern of cyanobacterial bloom and not algal bloom in the region. What does she think that prevent eukaryotic algae to bloom if they are present in the studied system and what does she think that prevents the cyanobacteria to proliferate?

Finally, and as general coment I miss reading a general discussion of the thesis. The topics included in her work are quite different but still is a pity there is no general discussion.

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In summary, the work of PhD candidate Hana Medová presents important and new results and proves her ability to conduct productive scientific research. The submitted doctoral thesis meets international standards. Hana Medová has proven her competence for doing multidisciplinary research, dealing with a wide range of topics while using cutting-edge approaches. I believe that she has the necessary talent and abilities to achieve a successful career in biosciences. For all these capacities, I can fully recommend Hana Medová to be awarded by the title *Doctor of Philosophy* Ph.D.

Barcelona, June 3rd 2013

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Review of the doctoral thesis of Mgr. Hana Medová entitled

Phototrophic microorganisms in extreme environments

The work of Mgr. Hana Medová conducts an ecophysiological study of phototrophic microorganisms in extreme environments. The submitted thesis has 123 pages and it is written in English. The thesis is based on one published, one submitted articles and two manuscripts.

The thesis represents a new data in terms of ecophysiological characteristics of phototrophic bacteria including anoxygenic phototrophic bacteria (APB). Especially, it was focused on the extreme environments and verified APB distribution and activity there at the first time. The thesis could be quite celebrated in this study field. Moreover, the presented data would provide a new feature and implication about ecophysiology of APB. A part of the results was already published in a respected international journal.

In this thesis, it is crutial for achieving the above-mentioned fruits to use the multiple physical and chemical techniques such as kinetic fluorometry, epifluorescence and light microscopy, emission spectroscopy, high performance liquid chromatography and the work with bacterial cultures. To master such a large number of experimental techniques it requires both perseverance and talent.

In spite of that I am obligated to raise some comments and questions:

Chapter 1

This chapter provides a brief description of the phototrophic organisms in extreme environment as a general introduction. In this introduction part, it is needed to summarize the previous studies about APB including aerobic anoxygenic phototrophs (AAPs) distribution. The focused standpoints in the Chapters 2 and 3 are about their distribution in extreme environments. So I recommend you to

compare your data to previous data in not only extreme environments but also not extreme environments (open and coastal ocean, estuary and freshwater). There is no description about the effect of the post-mining water in the Chapter 5. General introduction should make the background and purpose clear throughout the study. I require you to definitely specify the place of Chapter 5 in this thesis.

Chapter 2

This chapter provides a field study of APB in the 23 steppe lakes in the Transbaikal region (Russia), in Uzbekistan (Central Asia) and in the Crimean peninsula (Ukraine). This study verified the distribution of AAPs in all the studied steppe lakes, their high abundance and high BChl *a* turnover rates. This discovery suggested an important role of AAPs in terms of carbon cycle in extreme environments. In supplements: Figure S2.2, the low temperature (77 K) emission spectra could suggest the differences of photosynthetic organisms composition among the sampling sites. This technique would be very attractive as a new approach to clarify the distribution and composition of photosynthetic organisms in natural environments.

p. 22

'AAPs were shown to play an important role in the microbial community in the ocean, shelf seas and river estuaries'

- Why do AAPs play an 'important' role? Is it due to their high abundance? rapid growth rate? global distribution? Please explain in detail and show your opinion in this term.

p. 28

Please show the detection limit for BChl *a* as well as Chl *a*.

p. 29

This data about AAPs morphology should be very important and interesting on consideration about carbon cycle that AAPs drive in soda lakes. Sieracki et al.

(2006) suggested that the size of AAPs was about 2 times larger than total bacteria. This would imply that AAPs play an important role to contribute carbon cycle. It should be important for making their importance clear to calculate their carbon biomass based on the size of AAPs in this study.

p. 30

Why did you divide lakes into three groups according to their total depth? Please explain your expectation. In another word, if the depth changes, what environmental conditions also change?

Mixing depth? Nutrient supply? Light condition?

p. 31

The data from Figures 2.3 and 2.4 showed a quite clear distribution pattern of APB controlled by environmental factors (oxygen, salinity, HS^-). It is quite striking. Sift change of oxygen concentration should cause the change of APB distribution pattern. What do you think about this vertical change of APB distribution in terms of physiological and phylogenetical characteristics of APB?

p. 33

'An important question which arises from the performed study is whether the observed BChl *a*-containing cells can be classified as purple non-sulfur bacteria or AAPs'

-Do you think whether it is necessary to assess the phylogenetic determination of APB using molecular techniques (denaturing gel gradient electrophoresis, cloning, metagenome analysis) in such as this study, or not? In another word, what can the use of these techniques enable to clarify?

p. 33 misspelling

prevously → previously

p. 34 misspelling

Nevetherless → Nevertheless

p. 34

'This supports the hypothesis that the local phototrophic community lives mainly photoheterotrophically by recycling organic matter rather than photoautotrophically by conducting CO_2 fixation'

- It would be striking hypothesis. This hypothesis could give us a new feature of phototrophic organisms in the specific extreme environments.

p. 35

What do you think about the difference of BChl *a* concentration detected by kinetic fluorometry between Khilganta and Doroninskoe lakes? The difference seems to be very large.

p. 36

It would be needed to do statistical analysis about correlation between APB abundance and salinity. If it is possible, please analyze with dividing on each lake. Depend on lake, it seems that the correlation patterns are different.

Chapter 3

This chapter provides a field study of AAPs in polar freshwater lakes on Svalbard and James Ross Island. This study verified the distribution of AAPs in two thirds of studied lakes. In addition, AAPs were more abundant in lakes with higher total nitrogen, Chl *a*, oxygen concentration and temperature. Although their numbers were limited in the lakes with a temperature lower than 5° C, AAPs appear to have adapted to the conditions present in the polar environments. Little is know about AAPs in polar region, so this study could be appreciated in this study field.

In this chapter, the word to mean 'aerobic anoxygenic phototrophs' was not unified; AAPs, AAP and AAP bacteria. Please unify them.

p. 52 and p. 54

Please describe the unit for bacterial abundance.

p. 54

'The abundance of APB bacteria seems affected by temperature (Figure 3.2). APB were more ~'

-The result and discussion about total prokaryote are missing. Please add.

p. 54

'This is in accordance with studies reporting that higher temperature and higher Chl *a* concentration implied a higher AAP abundance'

-What does this fact suggest about AAP's ecophysiological characteristics? Can you comment on that?

Chapter 4

This chapter represents a quite interesting data on the consideration to the long evolution of APB. This work could occur an interesting feature that the purple reaction center stability under high temperature largely depend on tertially structure stabilization through weak interhelical van der Waals interaction (structure-based stabilization). It is very impressive that the reaction centers of purple anoxygenic bacteria have a thermostability of primary charge separation. This study would be quite valuable for understanding the evolutional history of phototrophic bacteria.

-What do you think about the thermostability of predominant cyanobacteria's reaction centers in the tropical and subtropical oceans? Only one cyanobacterial strain from freshwater was conducted in this experiment.

-What do you think about the photosynthetic pigments concentration and functional absorption cross section of photosystem under higher temperature? Photosynthetic pigments including Chl, BChl and carotenoids might be decomposed by higher temperature even if the surrounding protein complexes protect these pigments.

p. 61 misspelling
posses → possess

p. 76 misspelling
expoloited → exploited
ingeneering → engineering

In this chapter, some references are not entirely described in reference part.
Kibayashi et al. (2005), Watson et al. (2005), Kimura et al. (2009)...
In addition, Kibayashi et al. (2005) → Kobayashi et al. (2005)

Chapter 5

This study estimates the effect of post-mining acidic and high-conductivity water on selected cyanobacteria and the possibility of cyanobacterial water bloom in the newly formed Medard Lake by means of Chl fluorometry. This study could clarify that the response of cyanobacteria to spoil-heap waters appeared to be species-specific and can promote selection of those resistant to post-mining environments. The actual water chemistry of the upper layers of Lake Medard did not prove to effectively prevent any cyanobacterial bloom.

-What do you think about the characteristics of the studied post-mining waters from each site in terms of toxic and metal stress? Now, it might be difficult to discuss about the differences of the effects of spoil-heap water samples on cyanobacterial PSII among the studied sites, because of unidentified water characters in terms of chemicals.

p. 95 and p. 113
'Low pH itself does not reduce photosynthetic activity'
'the low pH of spoil-heap water inhibited the cyanobacterial photosystem II'
-There are discrepancies between these sentences. Is the most critical reason of the inhibition the low pH of spoil-heap water?

Chapter 6. Thesis Summary

In the end of this thesis, author can definitely show the place of the presented studies in the framework on the whole study field and discuss the main results throughout this study. Unfortunately, there is no general discussion in this thesis. In addition, this thesis would provide a lot of impressive implication and perspective for the future study. So it is better to summarize them for the future researchers.

In summary, the work of Mgr. Medová presents important and new results and proves her ability to conduct productive scientific research. The submitted doctoral thesis meets international standards and provides a lot of impressive implication and perspective for the future study. I can fully recommend Mgr. Hana Medová to be awarded by the title *Doctor of Philosophy* Ph. D.

Trebon, May 31th, 2013

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