PhD-Thesis: **Towards understanding hydrogenase maturation in *Thiocapsa roseopersicina***

Written by: **Barna D Fodor**

Institute of Biophysics, BRC, Hungarian Academy of Sciences, Szeged

and

Department of Biotechnology
University of Szeged
Hungary

In general, this is a very good PhD-thesis based on interesting and well performed experiments. The overall theme, microbial hydrogenases and their capacity to evolve and take up molecular hydrogen (H_2) is of great interest. H_2 is, according to recent scientific advances in the utilization in fuel cells and opinions expressed by both industrial and governmental representatives, the future energy carrier. Good fundamental science addressing the basic question if biology can offer a solution to how the H_2 can/will be produced without the releaese of e.g. green house gases and using never ending resources such as solar energy and water is of great interest. The present thesis addresses this issue by examing the phototrophic bacterium *Thiocapsa roseopersicina*, its enzymes directly involved in H_2-metabolism and their specific maturation process. In addition, in the work presented, several more general molecular methods have been developed for this specific bacterium (e.g. a conjugation based gene transfer method, a transposon based mutagenesis system, a broad-host-range expression vector system) – most important tools for further molecular characterizations of *Thiocapsa roseopersicina*.

In short, the candidate (Barna Fodor) has performed high level fundamental science on a biological system with great potential (directly or as a model system) for the future which may even have real implications on future societies. The techniques used, and the ones developed, are most appropiate and are internationally up-to-date. Obtained results are impressive and the conclusions drawn (including future prospects of the work) are based on solid, correctly interpretated data. The publications are sufficient for obtaining a PhD, and the language (as well as the overall impression) of the thesis is very good.

The key findings/accomplishments in the thesis are:

1. The development of a conjugal based gene transfer system for *Thiocapsa roseopersicina*.
2. The developemnt of a transposon based mutagenesis system for *Thiocapsa roseopersicina*.

Comment (1 & 2) – Fundamental techniques needed for further molecular characterizations of this particular bacterium.

3. The design, construction and use of broad-host expression vecorts with various affinity tags.

4. The production of a miniTn5 mutant library of *Thiocapsa roseopersicina.*

Comment (3 & 4) – Fundamental tools needed for further molecular characterizations of this particular bacterium

5. The identification of *hypF*, and the construction and characterization of a *hypF*-minus-mutant.
6. The deletion of *isp1* and *isp2* from the *hynS-isp1-isp2-hynL* operon of *Thiocapsa roseopersicina* as well as an initial chacterization of the mutant.

Comment (5 & 6) – Most interesting molecular results obtained when analyzing structural genes as well as an accessory gene involved in the maturation process.

7. The demonstration of an interaction between HypC2 and HynL during the maturation process.
8. The demonstration that HupK interacts with GroeEL.

Comment (7 & 8) – Most interesting biochemical results obtained when analyzing accessory gene products involved in the maturation process.

Questions. In general, I like the thesis, obtained data, the presentation and the discussion and have, therefore, very few "critical questions". However, when reading through the thesis I noted the following:

1. *Thiocapsa roseopersicina* harbours several NiFe-hydrogenases. What other enzymes are known in *Thiocapsa roseopersicina* also containing Ni? Any information about their accessory gene or gene products?

2. There is a single mRNA species coding for the *hynS-isp1-isp2-hynL* operon. Any indication of several transcripts? Isp1 and Isp2 have been demonstraded in the laboratory of the candidate, speculation(s) about the biological function(s) of these two gene products? Interestingly, Isp1 and Isp2 were found not to be required for the *in vitro* activity (as well as the maturation of HynSL). Any attempts to generate either *isp1* or *isp2* mutants? RT-PCR experiments using developed mutants?

3. What were, in the candidates hands, the most difficult parts when developing (a) the conjugation based gene transfer system, and (b) the random transposon mutagenesis method?

4. When emplying developed tools and methods to purify proteins from *Thiocapsa roseopersicina* some drawbacks were encountered regarding e.g. amount and purity. Suggestions to overcome these problems?

5. I find the observed interaction between HupK and GroEL very interesting. Suggestions how to continue to analyze this important observations?

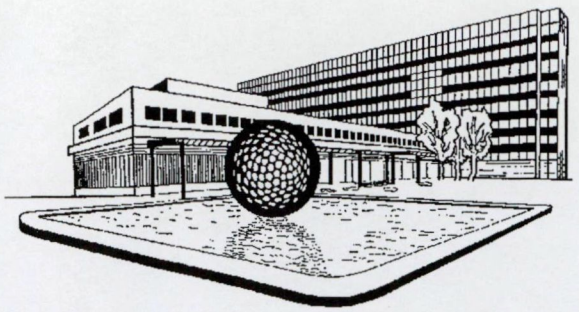
In conclusion, this is an internationally very good PhD-thesis. It is based on solid data which are presently and dicussed in a correct manner. The scientific level is high and I can only congratulate the candidate, Barna Fodor, the supervisors and the University

for a major accomplishment. My suggestion for a final ranking is "summa cum laude" (excellent).

Uppsala October 26, 2003

/Dr Peter Lindblad/
Professor in Biology
Dept Physiological Botany, Uppsala univ, Villavägen 6, SE-752 36 Uppsala, Sweden

Tel & Fax: +46-184712826; Tel (mob): +46-70-4250498
E-mail: Peter.Lindblad@ebc.uu.se



Zoltán Gombos D.Sc.

INSTITUTE OF PLANT BIOLOGY
BIOLOGICAL RESEARCH CENTER
Hungarian Academy of Sciences
H-6701 Szeged, Temesvári krt. 62., P.O.B. 521,
Hungary
Phone: +36-62-432-232
e-mail: gombos@nucleus.szbk.u-szeged.hu

OPPONENT'S COMMENT

The Ph.D. thesis written by Barna D. Fodor entitled "Towards understanding hydrogenase maturation in Thiocapsa roseopersicina" is a wellconstructed and grammatically correct thesis. I shall give my general and specific comments in the order of chapters in the thesis.

1. General comments.

a) The subject of the thesis is in the front of the research on the field. Nowadays, to satisfy the energy request of human civilization is one of the main problems. An alternative energy source based on H_2 is one promissing (promising) solution of the future energy supply.

b) The thesis is well constructed and the individual chapters are proportional. Since the logic of the thesis is clear it can be easily followed and it is easy to read.

c) The number and the quality of the referred publications demonstrate that the author knows the main lines of his subject and he is able to summarize the results of his field.

d) The used techniques and methods are modern, appropriate and well illustrated in the chapter of Materials & Methods. The description of the individual techniques are detailed enough to reproduce the method.

e) The style and the language of the dissertation are excellent and I could find just a few mistakes that I indicated in the text. The figures are demonstrative and well constructed.

f) The thesis satisfies the communication requests of the university. It is based on 3 publications. Two of them have already been published, one is submitted to

internationally well quoted journals. The candidate is first author in two of these papers, which is a basic requirement. Additionally he has other 4 publications.

2. Specific comments.

a) The "Introduction" is a nicely illustrated chapter. When I try to criticize the text I had to work hard. Some very minor comments;

   i. The title of chapter 1.2.2.1. is a little misleading. The candidate mentions a very specific protease in the text but on the basis of the title one might think that this chapter is about proteases in general.

   ii. Structurally it would be better to move chapter 1.4. before 1.3.

b) The "Aim of Study" assigns the aims of the work. It is short but well constructed

c) The "Materials & Methods" is well written and illustrated chapter.

d) The "Results & Discussion" is the best pat of the thesis. It is a concise, well written and logic part of the work. I have just some comments on the structure but these do not affect the very high quality of this chapter:

   i. The Fig. 5. should go before the description of Fig.6.

   ii. It would be better to change page no 49 to 48.

   iii. The labels of individual proteins are missing on Fig. 11 (p. 60.).

   iv. The unit is missing in Table 4.

e) The "Summary" well emphasizes the most important results of the dissertation.

3. In my opinion, the new and important results of the thesis are as follows:

i. A conjugation-based gene transfer was carried out in a photosynthetic organism.

ii. The candidate did the first transposon mutagenesis in *T. roseopersicina* opening a new gate in the genetics of these organisms.

iii. The construction of a broad-host range expression vectors.

iv. With the use of pMHE* vectors he demonstrated that with strain-specific promoters they can be used for overexpression and purification of proteins.

v. He developed a technique for studying protein-protein interactions.

vi. The production of a mutant library of *T. roseopersicina*.

vii. Identification of the hypF gene.

viii. Heterologous complementation experiments evidenced that the hypF gene is interchangeable among bacteria, however, even if the complementation was not complete.

ix. The demonstration that certain gene products which were suspected to play an important role in the maturation of HynSL hydrogenase are not essential in that process.

x. The presentation that specific protein-protein interactions determine the maturation process.

xi. The putative role of GroEL.

I found that B. Fodor's results, summarized above, are important achievements of the "hydrogenase world". I find that his thesis meet the requirements of the Ph.D. process, therefore I suggest that following a successful defense to be awarded the Ph.D. title.

Zoltán Gombos