Ph.D. THESIS

RELATIONSHIP BETWEEN PHA AND HYDROGEN METABOLISM IN A PURPLE SULFUR PHOTOTROPHIC BACTERIUM

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Introduction

In the near future, the increasing energy demand of the human populations can not be supply by fossil energy sources which are being depleted.

The alternative energy carriers replacing fossil energy should be produced from renewable sources in large scale; they should be enviromental friendly, storable etc. The most promising candidate is the really “green” hydrogen, of which oxidation result pure water as by-product.

Hydrogen can be produced by chemical or biological processes. In the biological hydrogen production, two main enzyme groups are involved: hydrogenases and nitrogenases.

Hydrogenases are ancient enzymes catalyzing the simplest reaction in nature, the oxidation of hydrogen and/or reduction of protons.

The other alternative enzymes for hydrogen production are nitrogenases. They convert the molecular nitrogen to ammonia satisfying their and other organisms’ nitrogen demand. During this process large amount of hydrogen is produced.

The phototrophic bacteria, under proper growth conditions, are able to accumulate several storage materials, such as elementary sulphur, glycogen, polyhydroxyalkanoates (PHAs) and polyphosphates.

The accumulated PHAs serve as carbon and energy storage materials in many bacteria.

In bacteria, the PHAs have several biological roles: they are involved in stress endurance, plant- microbe interactions and can be an energy source for several cellular processes

The PHAs as biodegradable plastics are also considered for industrial applications (medicine, agriculture, packaging and food industry), but under specific circumstances their “reducing power content” can also be used.

Thiocapsa roseopersicina BBS is a purple sulfur phototrophic bacterium had an extrem metabolic versatility: it can utilize several substrates and accumulate various storage materials.

Our group's studies revealed, that the *T. roseopersicina* hydrogenases and nitrogenase are connected to several metabolic pathways (sulfur, glycogen, glucose, acetate etc.) and the electrons coming from these metabolic pathways can be used for hydrogen production.

Aims

In the phototropic bacteria, the accumulated PHAs are mainly carbon and energy sources, but under certain circumstances PHA can be used for hydrogen production.

PHA is accumulated during nutrient starvation such as nitrogen or phosphorous limitations. In my work, nitrogen fixing conditions were used leading to nitrogenase mediated hydrogen evolution. In this case, hydrogenases reoxidize hydrogen for recovering energy for the cells, therefore in my experiments a strain lacking any hydrogenase activity was used.

The main aims of my work were:

- finding of the optimal medium for the best PHA accumulation.
- identification of the genes involved in PHA metabolism.
- disclosure of the role of polyhydroxyalkanoates in nitrogenase based hydrogen production.
- investigation of the effect of external electron donors (thiosulphate, succinate) on hydrogen production in the presence/ or absence of PHAs.

Methods

T. roseopersicina cells have been propagated in the presence of several carbon and nitrogen substrates to find the optimal nutrients and the best C/N ratio for efficient PHA accumulation. Using bioinformatical approches, all genes involved in polyesters metabolism were identified and characterized.

The standard DNA manipulations techniques were applied for construction of a PHA biosynthetic mutant (PH12B) in strain lacking active hydrogenase (DC12B). Plasmids were transferred into *Escherichia coli* by chemical transformation and into *T. roseopersicina* via conjugation.

The amount of hydrogen and PHA produced by the strains were quantified by gas chromatografy. The thiosulphate and succinate contents of the cells were measured by spectofotometric method and HPLC, respectively.

The *in vitro* nitrogenase activity was followed by acetylene reduction assay.

Results

In this study the following results were achieved:

I. Among various substrates, acetate (10 g l^{-1}) supplemented with 0.17 g l^{-1} glutamate was found to be the best carbon source for PHA accumulation. Under these conditions *T. roseopersicina* was able to accumulate PHA up to 33 % of the dry cell weight.

II. Using the *T. roseopersicina* genomic database I have identified and characterized the genes involved in PHA biosynthesis (*phaBPRACE*) and degradation (*phaZ*).

III. A comparison of the phylogenetic trees of the PhaC and PhaZ proteins revealed significant differences, which indicated their different evolutionary histories and a possible horizontal gene transfer event.

IV. In order to test the role of polyesters in hydrogen metabolism, I prepared a PHAs biosynthetic mutant strain (PH12B) on a hydrogenase free background (DC12B). In the PH12B strain, no PHAs accumulation could be detected according to my expectations.

V. I optimized a dual-stage efficient hydrogen production system for *T. roseopersicina,* where the PHA accumulating growth conditions and H_2-producing phases are temporally separated.

VI. I demonstrated that the stored PHAs are good substrate for H_2 production. Comparing the hydrogen production of *Δpha* and its corresponding parenteral strain, significantly higher H_2 production could be observed in the presence of polyesters which correlated well with the increased PHB degradation rate.

VII. I found that the addition of primary electron sources (thiosulphate and/or succinate) to the H_2-producing medium substantially increased the H_2 production in both strains. This effect might be due to the multiple role of thiosulphate capable of: i) feeding electrons to the nitrogenase via quinone

pool, ii) enhancing the *in vitro* nitrogenase activity, iii) stimulating the PHA degradation via an unknown mechanism.

Publications

Publications covering the thesis

Fülöp, A., Béres, R., Tengölics, R., Rákhely, G., Kovács, K.L. (2012) Relationship between PHA and hydrogen metabolism in the purple sulfur phototrophic bacterium *Thiocapsa roseopersicina* BBS. Accepted for publication: *Int J Hydrogen Energy.*
IF: 4,053

Fülöp, A., Rákhely, G, Kovács, K.L. Polyhydroxyalkanoates role in hydrogen production in a purple sulphur bacteria. Poster presentation: 7th European Workshop on Bacterial Respiratory Chains, May 11-15, 2011, Backagarden, Sweden.

Rákhely, G., Béres, R.**, Fülöp, A.,** Latinovics, D. and Kovács, K.L. Connection between hydrogen metabolism and biopolymer storage materials in purple photosynthetic bacteria. Poster presentation: Biotechnical Functionalisation of Renewable Polymeric Materials COST 868 meeting. April 17-18, 2008, Bratislava, Slovakia.

Other publications

Kiss, I., Oskolás, H., Tóth, R., Bouillet, P., Tóth, K., **Fülöp, A.,** Scholtz, B.,. Ledent, C.A., Fésüs, L. and Szondy, Z. (2006) Adenosine $_{A2A}$ receptor-mediated cell death of mouse thymocytes involves adenylate cyclase and Bim and is negatively regulated by Nur77. *Eur J Immunol.* **36:**1559-1571
IF: 5,005

Kovács, Á.T., Rákhely, G., Browning, D.F., **Fülöp, A.,** Maróti, G., Busby, S.J.W., Kovács, K.L. (2005)**.** An FNR-type regulator controls the anaerobic expression of Hyn hydrogenase in *Thiocapsa roseopersicina. J. Bacteriol*. **187**(8):2618-2627.
IF: 4,175

Kovács, Á.T,, Rákhely, G., Balogh, J., Maróti, G., **Fülöp, A.,** Kovács, K.L. (2005) Anaerobic regulation of hydrogenase transcription in different bacteria. *Biochem Soc Trans* **33**(1):36-38.
IF: 2,579

Kovács, K.L., Kovács, Á.T., Maróti, G., Mészáros, L.S., Balogh, J., Latinovics, D., **Fülöp, A.,** Dávid, R., Dorogházi, E., Rákhely, G. (2005) The hydrogenases of Thiocapsa roseopersicina. *Biochem Soc Trans* **33**:61-3.
IF: 2,579

Kovács, K.L., Kovács, Á.T., Maróti, G., Bagi, Z., Csanádi, Gy., Perei, K., Bálint, B., Balogh, J., **Fülöp, A**., Mészáros, L.S., Tóth, A., Dávid, R., Latinovics, D., Varga, A., Rakhely, G. (2004) Improvement of biohydrogen production and intensification of biogas formation. Rev Environ Sci & Bio/Technology 3:321–330.
IF: 0,876

Kovács, K.L**, Fülöp, A.,** Herbel, Z., Nyilasi, A., Rákhely, G. (2010) Tiszta, megújuló energia a biohidrogén, Környezetvédelem **XVIII.(2)**:20-21.

Posters

Béres, R., **Fülöp, A.,** Szász, I., Kovács, L.K., Zinn, M. & **Rákhely, G.** *Biopolymers in phototrophic bacteria: special bioplastics and source of biofuels*. 7th International Conference on Polymer and Textile Biotechnology, 02-04 March 2011, Milan, **Italy**.

Rákhely, G., Balogh, J., Balogh, T., **Fülöp, A.**, Győri, E., Latinovics, D., Palágyi-Mészáros, L.S., Novák, J., Tengölics, R. & Kovács, K.L. *Synthesis and utilization of biopolymers in purple photosynthetic bacteria*. First annual workshop of the COST 868 Action on Biotechnical functionalisation of renewable polymeric materials, 12 -14 September 2007, Graz, **Austria**.

Fülöp, A., Kovács, Á.T., Klem, J., Rákhely, G., Kovács, K.L. *The function and regulation of the fnrt gene in the purple sulfur photosynthetic bacterium*. Straub Days, Biological Research Center, 15-17 November 2006, Szeged, **Hungary**.

Fülöp, A., Kovács, Á.T., Klem, J., Rákhely, G., Kovács, K.L. *The function and regulation of the fnrt gene in the purple sulfur photosynthetic Thiocapsa roseopersicina BBS.* Modern Trends in Biological Sciences: Seeking an Integrative approach 1st ITC Alumni Meeting, Biological Reserch Center, 19-20 October 2006, Szeged, **Hungary**.

Maróti, G., Bagi, Z., Rákhely, G., Takács, M., Tóth, A., Kovács, Á.T., Bálint, B., Mészáros, L.S., Balogh, J., **Fülöp, A.**, Latinovics, D., Dávid, R., Dorogházi, E., Nyilasi, A., Kovács, K.L. Biohydrogen, biogas. Biovision, 11-15 April 2005, Lyon, **France.**

Kovács, Á.T, Rákhely, G., Browning, D.F., **Fülöp, A.**, Maróti, G., Busby, S.J.W., Kovács, K.L. *An FNR-type regulator controls the anaerobic expression of Hyn hydrogenase in Thiocapsa roseopersicina*. Biological Reserch Center Days, 7-9 December 2004, Szeged, **Hungary**.

Kovács, Á.T., Rákhely, G., Browning, D.F., **Fülöp, A.**, Maróti, G., Busby, S.J.W., Kovács, K.L. *An FNR-type regulator controls the anaerobic expression of Hyn hydrogenase in Thiocapsa roseopersicina*. International Hydrogenase Conference, 24-29 August 2004, Reading, **United Kingdom**.

Kovács, Á.T, Rákhely, G., **Fülöp, A.**, Browning, D.F., Busby, S.J.W., Kornél, K.L. *FNR, anaerobic regulator in a photoroph bacterium* (in Hungarian). Conference of the Hungarian Society of Biochemistry, 10-13 May 2004, Sopron, **Hungary**.

Kovács, Á.T., Rákhely, G., Maróti, G., Balogh, J., Latinovics, D., **Fülöp, A.**, Busby. S., Kovács, K.L. *Regulation by light, oxygen and hydrogen in a purple sulfur photosynthetic bacterium*. Conference on Molecular Microbiology: Exploring Prokaryotic Diversity, 22-26 April 2004, Heidelberg, **Germany**.

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