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DISSERTATION SUMMARIES

Investigation of the relationship of hydrogenase enzymes and photosynthesis in *Thiocapsa roseopersicina*

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The photosynthetic purple sulphur bacterium, *Thiocapsa roseopersicina* harbours four functional [NiFe] hydrogenases. Two of them are attached to the periplasmic membrane (Hyn, Hup) and the other two are apparently localized in the cytoplasm (Hox1, Hox2). It prefers to utilize reduced sulphur compounds for anaerobic photochemolithotrophic growth, but simple organic substrates such as glucose and acetate can also be used as carbon, energy and electron sources.

There is a facultative lithoautrophic proteobacterium, *Ralstonia eutropha* which has a soluble hydrogenase gene, *hoxI.* In *R. eutropha* the HoxI protein has an established role in the *in vivo* photosynthetic electron transport similar to that of the Hox1E in *T. roseopersicina*. The in frame deletion of *hox1E* gene causes complete loss of *in vivo* hydrogenase activity in *T. roseopersicina* and in addition, this loss-of-function mutation was not complemented by *hoxI* gene.

Various constructs harbouring the *hoxI* gene were transferred into different *T. roseoperscina* hydrogenase mutant strains.

Functional studies aimed the investigation of the role of *hoxI* in the modulation of Hox1 enzyme. Therefore, experiments were carried out under conditions when only Hox1 hydrogenase was functional (elevated sodium-thiosulphate concentration in the medium). The strain expressing the *hoxI* gene was shown to evolve significantly higher amount of hydrogen *in vivo* compared to the control (empty plasmid in the same strain).

The global gene expression changes were studied in three different strains by sequencing-based Whole Transcriptome Analysis (WTA): the strain which contains the complete Hox1 hydrogenase, the *hox1E* mutant strain and the *hox1E* mutant strain harbouring the heterologous *hoxI* gene.

The WTA results were divided into two groups. The first group deals with the genes, which showed decreased expression in response to *hox1E* deletion (two genes organized into one operon and coding for the NADH dehydrogenase subunit 5 protein and a hypothetical transmembrane protein coupled to NADH-ubiquinone oxidoreductase chain 5 homolog). The low expression levels of these genes were not complemented by the *hoxI* gene. The second group represents genes, which products are involved in the organization of the photosynthetic reaction center and the light harvesting complex. These genes also showed reduced expression in the *hox1E* mutant strain, however the addition of the *hoxI* gene restored their expression levels.

Currently we are investigating the metabolic background of the gene expression changes by deploying different molecular and functional experiments as well as mutant analysis studies. Light dependence of the Hox1 hydrogenase is under investigation, this might shed light on the interaction of Hox1 hydrogenase and photosynthesis.

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