Connection between the membrane electron transport system and Hyn hydrogenase in purple sulfur bacterium, *Thiocapsa roseopersicina* BBS.

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Introduction.

Thiocapsa roseopersicina BBS is an anoxygenic, photosynthetic purple sulfur bacterium. Various sulfur compounds (sulfide, sulfur, thiosulfate) are used as electron donors and carbonate as inorganic carbon source for growth. The thiosulfate assimilation take place via Sox cycle. Sulfur globules are formed as intermediate product of thiosulfate, exogenous sulfur and sulfide assimilation. Elementary sulfur can be oxidized to sulfite via sulfide by the DSR complex. ***Thiocapsa roseopersicina* can produce hydrogen under various conditions with its [NiFe] hydrogenases**.

IN THIS WORK WE AIMED TO DISCLOSE THE ELECTRON TRANSPORT BETWEEN HYN AND THE MEMBRANE REDOX SYSTEM.

About the Hydrogenases:

Hydrogenases can catalyze the reversible reduction of protons.

$$H_2 + A_{ox} \longleftrightarrow 2H^+ + A_{red}$$

The hydrogenases of *T. roseopersicina*:

In this microorganism. two soluble NAD^+-reducing (Hox1 and Hox2) and two membrane-bound (HupSL and HynSL) hydrogenases were identified. **Hox1 is connected to storage materials, carbohydrate metabolism and produces hydrogen in the presence of reduced sulfur compounds.** Hox2 produces hydrogen in the presence of glucose, Fig.1.

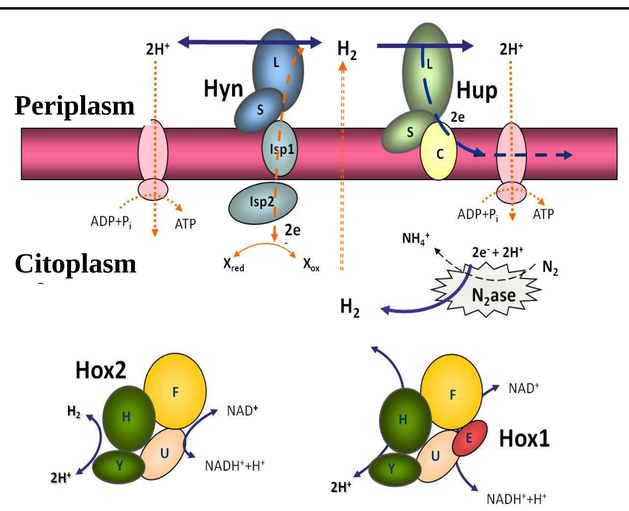
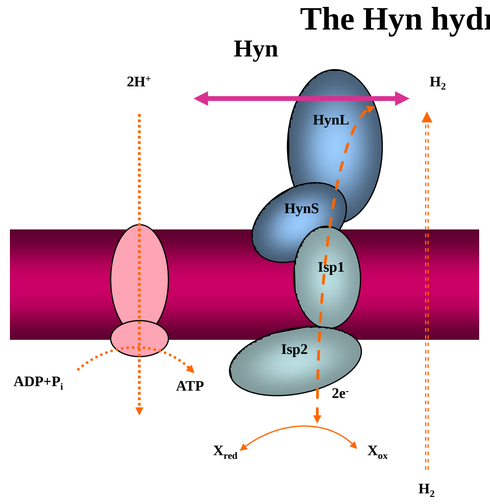


Fig.1.: The hydrogenases in *T. roseopersicina*

The Hyn hydrogenase



- Bidirectional, membrane bound enzyme
- **Isp1 and Isp2:** two **electron transfer subunits**.

Fig.2.:The model of the Hyn hydrogenase

Strains:

GB2131 (ΔHox1, Δ HupSL), The wild type Hyn hydrogenase is the only active enzyme under our experimental conditions.

pTHOE5M (Δ HynSL, Δ Hox1, Δ HupSL + HynSL) contains recombinant Hyn hydrogenase.

Isp2M (ΔHox1, Δ HupSL), wild type Hyn without, Isp2 electron transport subunit.

Hydrogen evolution of Hyn

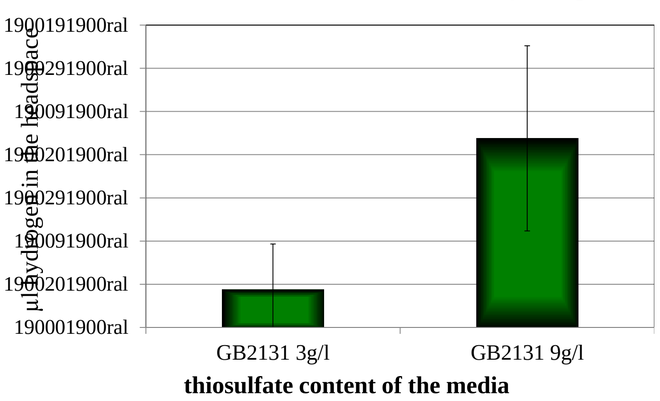


Fig.3 Hydrogen evolution of GB2131 in the presence of different amount of thiosulfate.

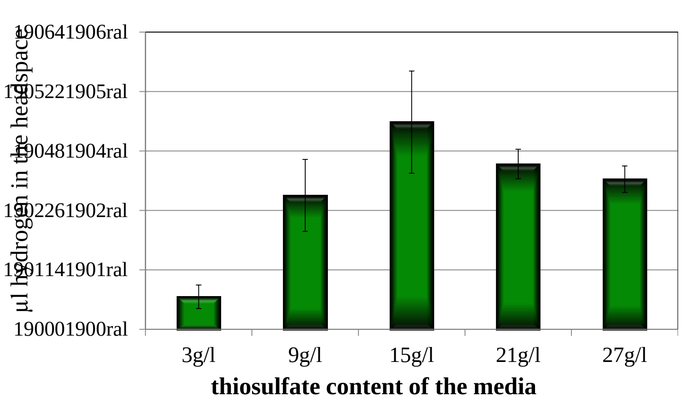


Fig.4. Hydrogen evolution of pTHOE5M in the presence of different amount of thiosulfate.

The elevated concentration of sodium thiosulfate increased the hydrogen evolution of GB2131 and pTHOE5M strains, therefore hydrogen production of Hyn hydrogenase can be driven by sodium-thiosulfate.

Hyn hydrogenase linked to sulfur metabolism

Electron donors of hydrogen evolution of Hyn

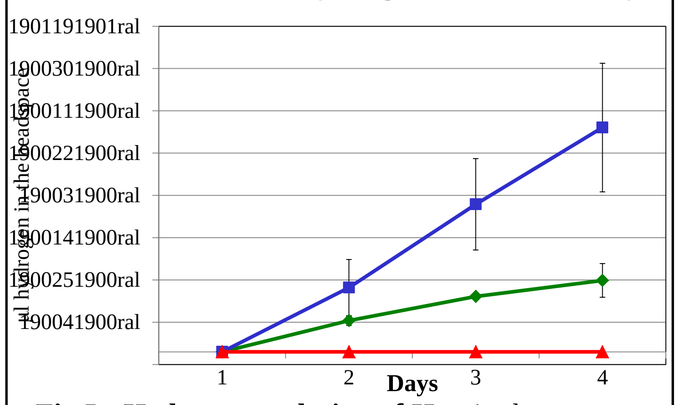


Fig.5.: Hydrogen evolution of Hyn in the presence of stored elemental sulfur (■); stored elemental sulfur + thiosulfate (◆) and sulfite (▲). The pTHOE5M strain was used under illumination

Oxidation of elemental sulfur and thiosulfate assimilation provide electrons for the hydrogen evolution of Hyn. While sulfite is not electron donor of Hyn. (Fig 3;4;5)

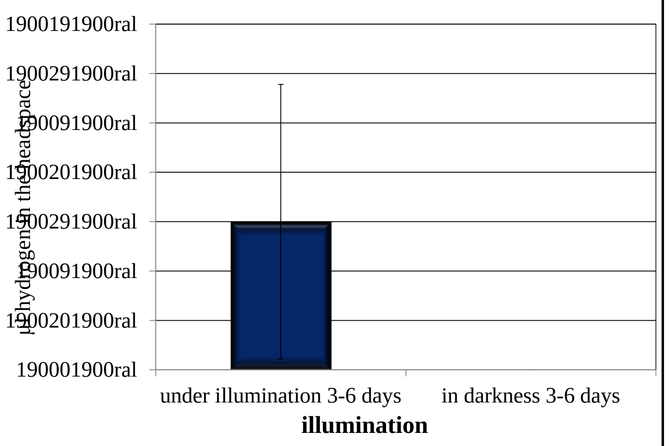


Fig.6 Light dependence of hydrogen evolution of Hyn between 3-6 days (GB2131 strain).

Electron transport subunit of Hyn

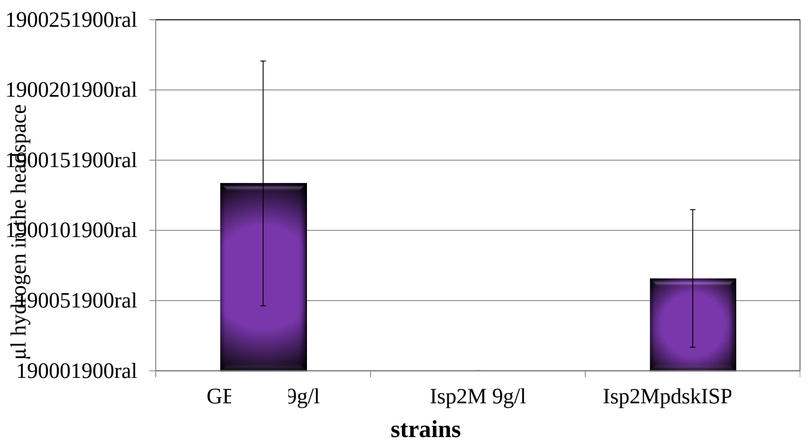


Fig.7.: Hydrogen evolution of Hyn in the presence of 9 g/l sodium thiosulfate and **in the absence of Isp2** subunit of it**.**

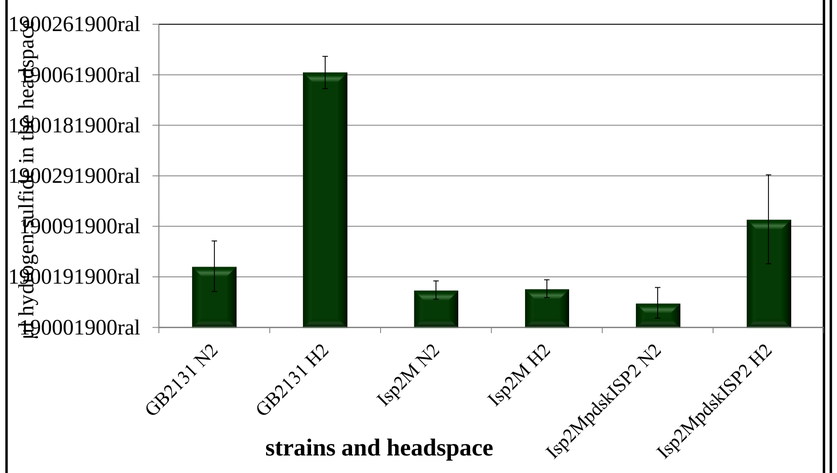


Fig. 8: Hydrogen sulfide formation of GB2131 and Isp2M strains under nitrogen and hydrogen atmosphere. Initial elemental sulfur content was 12,4 mM

The link between the central redox procesess and HynSL is the membrane associated cytoplasmic Isp2 subunit of Hyn in both directions.

Electron acceptor pathway of Hyn

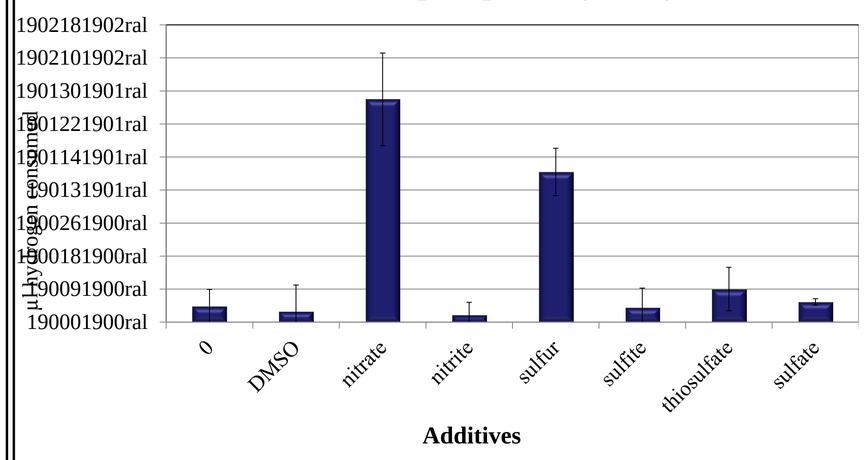


Fig.9.: Hydrogen uptake of Hyn in the presence of different kind of potential electron acceptors (GB2131 in darkness).

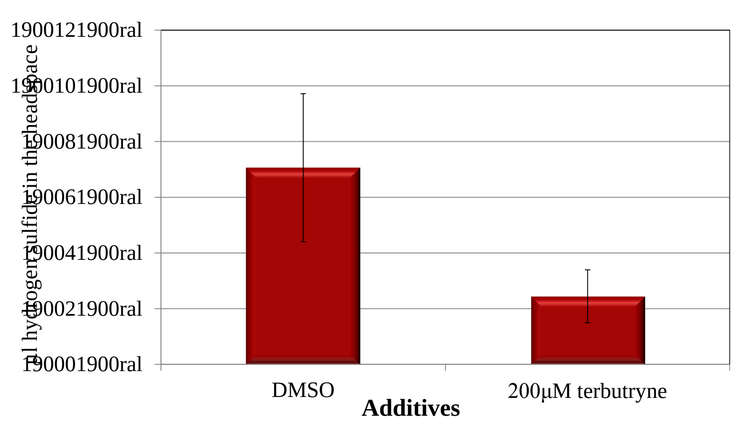


Fig.10.: Hydrogen and Hyn hydrogenase linked hydrogen sulfide formation of GB2131 strain in darkness **in the presence** and absence of - Q_b site competitive electron transport inhibitor – **terbutryne.**

Q_b site of photosynthetic reaction center is the part of the electron transport chain between Hyn and membrane quinon pool.

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CONCLUSIONS

According to our data an integrated - but still hypothetical - metabolic model for Hyn could be outlined. The oxidation of sulfur and thiosulfate have an important role in hydrogen evolution of Hyn and this process is light driven. There is a bidirectional connection between Hyn hydrogenase and membrane redox system via the Isp2 electron transfer subunit of Hyn and Q_b site of RC in the photosynthetic membrane.