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Examination of the hydrogen-metabolism in *Methylococcus capsulatus* (Bath)

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Methylococcus capsulatus (Bath) is a Gram-negative, methylotrophic bacterium, which oxidizes methane to carbon dioxide for energy generation. The enzyme complexes methane monooxygenases (MMOs) oxidize methane to methanol and co-oxidize a wide variety of aliphatic, aromatic and halogenated hydrocarbons, therefore they are extremely versatile enzymes for biocatalysis and bioremediation. The in vivo electron donor of the MMOs is NADH, which must be regenerated. Since biodegradation processes using MMO are co-oxidation processes, alternative ways of supplying reducing power are needed. Possible candidate could be H_2 for NADH + H^+ generation.

Hydrogenases are metalloenzymes catalyzing the reversible oxidation of H_2. *M. capsulatus* (Bath) contains a soluble (Hox) hydrogenase - which is able to reduce NAD^+ using H_2 - , and a membrane-bound nickel-iron Hup hydrogenase - which plays an important role in the recycling of hydrogen, and maybe donates the electrons to the quinone pool. Another enzyme – nitrogenase - produces H_2 as a byproduct under nitrogen fixing condition.

Δ*hupSL* and Δ*hoxH* deletion mutants were generated (Csáki et al. 2001). H_2-driven MMO activities of these mutants and wild type were measured to obtain information about the *in vivo* function of the hydrogenases (Hanczár et al. 2002). The deletion mutants revealed unexpected behavior: the Δ*hupSL* mutant did not show H_2-driven MMO activity, while the Δ*hoxH* mutant showed. The Hup hydrogenase - which is unable to reduce NAD^+ directly - is required for the H_2-driven activity of MMO. To understand the role of Hup hydrogenase in H_2-metabolism the first step is to find all genes coding for proteins, which has any affect on Hup hydrogenase activity.

Several Hup^- phenotype mutants were isolated from a *M. capsulatus* random mutant library, which was generated by transposon mutagenesis. The transposon was found in a structural gene (*hupL*), in an accessory gene (*hupD*) of Hup hydrogenase, and in other genes: TonB-dependent receptor-like putative protein coding gene (*tonB*) and conserved hypothetical protein for NADH ubiquinone/plastoquinone complex coding gene (*nupX*).

The *in vivo* H_2 production capacities of the wild type and the mutant strains were compared. The Hup hydrogenase of the wild type consumed a lot of H_2 from the gas phase, while the mutants had lower H_2 consumption activity both under nitrogen fixing and nitrogenase repressed conditions. Hup hydrogenase structural proteins were detected both in wild type, $HupD^-$, $TonB^-$ and $NupX^-$ transposon mutants with HupL antibody by Western Blot assay, in contrast to the Δ*hupSL* deletion and $HulpL^-$ transposon mutants.

The results show the presence of the matured Hup hydrogenase in $TonB^-$ and $NupX^-$ transposon mutants, but hydrogen-metabolism of these mutants is damaged, therefore they have Hup^- phenotype. According to the *in silico* analysis and global protein alignment the proteins of the *nupX* containing operon are similar to the NuoM, NuoL and NuoN proteins of the NUO (NADH ubiquinon-plastoquinon oxydoreductase) complex. In our hypothesis the proteins of the examined nuo-like operon maybe play a role in the energy conversion of the bacterium, while the examined TonB-dependent receptor-like putative protein perhaps takes part in the mechanism of TonB-catalyzed iron transport through the bacterial cell envelope, indirectly contributing to the assembly of the membrane-bound nickel-iron Hup hydrogenase.

To determine the role of the mutant genes further investigations are needed.

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