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DISSERTATION SUMMARY

Isolation of hydrogenase deficient mutants in purple sulfur photosynthetic bacteria by transposon mutagenesis

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Hydrogenases catalyze the reversible oxidation of molecular H_2. Two major classes are distinguished: Fe-only hydrogenases and [NiFe] hydrogenases. The latter is typically composed of an electron transfer small subunit and a catalytic large subunit. The formation of an active [NiFe] hydrogenase involves a complex maturation process. The major steps include the biosynthesis of the unprocessed, inactive subunits, incorporation of Fe-S clusters into the small subunit, the assembly of Ni, Fe and the diatomic ligands (CN, CO) into the active center in the large subunit, and the C-terminal cleavage of the large subunit by a specific protease. Without the action of the pleiotropic (Hyp) maturation proteins (HypA, HypB, HypC, HypD, HypE, and HypF) and the specific protease, maturation stops.

A conjugation based gene transfer system, site directed mutagenesis and random transposon mutagenesis system was optimized for the purple sulfur phototrophic bacterium, *Thiocapsa roseopersicina* BBS. This bacterium has at least three hydrogenases. Screening for hydrogenase deficient phenotypes resulted in the isolation of six independent mutants in a miniTn5 library. In the first class of mutants only the Hyn (hydrogenase 1) activity was affected. The second class of mutants had pleiotropic mutations affecting all hydrogenases. One of the pleiotropic mutations was in a gene showing high sequence similarity to HypF proteins in other organisms. This mutant was further characterized in *in vitro* and *in vivo* experiments. The reconstructed *hypF* gene was able to complement the *hypF* deficient mutant of *T. roseopersicina* BBS. Heterologous complementation experiments, using *hypF* $^-$ strains of *T. roseopersicina*, *E. coli*, and *R. eutropha*, and various *hypF* genes, were performed. Heterologous complementation was successful in all of the cases tested, although for the *E. coli* host the regulatory region of the foreign gene had to be replaced in order to achieve partial complementation. To characterize the products of the genes isolated, attempts to create a homologous expression system with affinity-tags were made. Expression of a reporter protein was demonstrated, and optimization of purification proce/ dures will be also presented.

References

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