Redirection of Metabolism for Hydrogen Production

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We focus on hydrogen production catalyzed by nitrogenase enzyme, as represented by the equation: $N_2 + 8H^+ + 8e^- + 16\ ATP > 2\ NH_3 + H_2 + 16\ ADP$. Nitrogenase generates pure hydrogen gas in situations where no nitrogen gas is available. Our experimental organism is the phototrophic bacterium *Rhodopseudomonas palustris*. *R. palustris* generates the ATP needed for hydrogen production from sunlight by cyclic photophosphorylation. And it obtains the electrons needed from organic compounds (which also serve as the carbon source for cells). It is assumed that the protons that end up in hydrogen gas are present free in water. In the initial stages of this project we completed a genomic and functional characterization of four newly sequenced strains of *R. palustris* (5). We selected the strain with the most robust hydrogen-producing abilities for further work. In initial work, we also developed some new gene expression vectors that were useful later in the project (6).

Our goal for this project was to elucidate pathways of electron flow from organic substrate to nitrogenase in *R. palustris* and to use the information so obtained to engineer improvements in hydrogen production. We worked with a mutant NifA* strain of *R. palustris* that synthesizes nitrogenase when supplied with ammonium as a nitrogen source and deprived of nitrogen gas. Such cells produce pure hydrogen gas during growth. During the project period we determined major pathways of electron flow from succinate, butyrate, fumarate, and acetate to nitrogenase when cells were making hydrogen gas (1, 4). This involved the use of ^{13}C metabolic flux analysis. We concluded that three factors influence the amount of hydrogen produced by cells. These are a) the reduction state of the organic compound (e.g, butyrate is much more electron rich than acetate), b) the amount of carbon dioxide-fixing Calvin cycle flux that competes against hydrogen production for electrons and c) the route that cells use to the metabolize the electron-donating carbon compound that they are provided with. Following from this work we found, as predicted, that we could improve H_2 yields by *R. palustris* cells by blocking electron flow to carbon dioxide fixation by mutagenesis (1, 4).

In related work, we showed that *R. palustris* can also use the inorganic compound thiosulfate as an electron source for both nitrogen fixation and hydrogen gas production (3). In addition we determined that hydrogen yields from *R. palustris* can be vastly improved by using nongrowing cells as biocatalysts, because during growth, most electrons from the donating organic compound are used for biosynthesis (2,3).

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Stephen Hawley (now a research scientist at the University of Minnesota)

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Publications

Journal articles:

1. McKinlay, J. B. and C. S. Harwood. 2011. Calvin cycle flux, pathway constraints and substrate oxidation state together determine the H_2 biofuel yield in photoheterotrophic bacteria**.** **mBIO**. March 22:2.

2. Gosse, J.L., B. J. Engel, J. C. Hui , C. S. Harwood, and M. C. Flickinger. 2010. Progress toward a biomimetic leaf: 4,000 h of hydrogen production by coating-stabilized nongrowing photosynthetic *Rhodopseudomonas palustris*. **Biotechnol Prog**. 26:907-918.

3. Huang J.J., E. K. Heiniger, J. B. McKinlay and C. S. Harwood. 2010. Production of hydrogen gas from light and the inorganic electron donor thiosulfate by *Rhodopseudomonas palustris*. **Appl. Environ. Microbiol.** 76: 7717-7712. [epub ahead of print].

4. McKinlay, J. B. and C. S. Harwood. 2010. Carbon dioxide fixation as a central redox cofactor recycling mechanism in bacteria. **Proc. Natl. Acad. Sci. USA** 107:11669-11675.

5. Oda Y., F. W. Larimer, P. S. Chain, S. Malfatti, M. V. Shin, L. M., Vergez, L. Hauser, M. L. Land, S. Braatsch, J. T. Beatty, D. A. Pelletier, A. L. Schaefer and C. S. Harwood. 2008. Multiple genome sequences reveal adaptations of a phototrophic bacterium to sediment microenvironments. **Proc. Natl. Acad. Sci. USA**. **105**:18543-18548. Epub 2008 Nov 1

6. Schaefer, A. L., E. P. Greenberg, C. M. Oliver, Y. Oda, J. J. Huang, G. Bittan-Banin, C. M. Peres, S. Schmidt, K. Juhaszova, J. R. Sufrin and C. S. Harwood. 2008. A new class of homoserine lactone quorum-sensing signals. **Nature** 454:595-599.

Review articles:

McKinlay, J. B. and C. S. Harwood. 2010. Photobiological production of hydrogen gas as a biofuel. **Curr. Opin. Biotechnol. 21**:244-251.

Book chapters:

McKinlay, J. B. and C. S. Harwood. 2011. Applications of stress response studies: biofuel production. IN: G. Storz and R. Hengge (eds). ***Bacterial Stress Responses*****, 2nd ed.,** Washington, D. C.: ASM Press.

Popular Press:

McKinlay, J. B. and C. S. Harwood. 2011. Harnessing Bacteria That Use Light To Produce Hydrogen. Microbe. August Issue.