155. Design Principles Controlling Hydrogen Metabolism in Phototrophic Organisms

Maria L. Ghirardi 1*(maria.ghirardi@nrel.gov) and Matthew Wecker2 (matt.wecker@nrel.gov)

1National Renewable Energy Laboratory, 15013 Denver West Parkway, Golden, CO 80401
2Genebiologics, LLC, Boulder, CO 80303
*Presenting author

Project Goals: To obtain a systems-level understanding of the biological barriers that control hydrogen metabolism and prevent sustained H2 photoproduction in the green alga Chlamydomonas reinhardtii.

Photobiological H2 production from water is a clean, non-polluting and renewable technology. Although the potential light conversion efficiency to H2 by biological organisms is theoretically high (about 10%), the system is currently limited by biochemical and engineering constraints. The specific objectives of this research are covered by two Tasks: (1) development, testing, validation and utilization of novel high-throughput assays to identify photosynthetic organisms with altered H2-producing activities, thus leading to the discovery of novel strategies to circumvent known biochemical limitations; and (2) deconvolution of the network of metabolic pathways centered on six ferredoxin homologs found in Chlamydomonas, aimed at understanding reductant flux in photobiological hydrogen production, and identifying targets for future metabolic pathway engineering strategies to reduce flux to non-productive pathways.

In Task 1, we developed, tested and validated a novel high-throughput assay to identify high H2-producing strains from an insertional mutagenesis library of C. reinhardtii. The assay uses the H2-sensing system of Rhodobactercapsulatus that responds to H2-production by algal colonies through activation of a GFP signal (Wecker et al., 2011). We validated this assay with well-characterized mutants that are either low or high H2-producers (Wecker and Ghirardi, 2014). Finally, using this H2-sensing system, we have isolated four insertional mutant strains of C. reinhardtii that exhibit high-light H2 production and have shown that these strains show up to 100-fold increased H2 production levels compared to their wild type strains when grown at elevated light levels. We are currently identifying the site of insertion and further characterizing these strains to understand which genes are responsible for these high-light H2-production phenotypes.

Concomitantly, we inserted a heterologous hydrogenase from Clostridium acetobutylicum into our R. capsulatus sensor strain. In doing so, we find that (i) hydrogen is produced by the heterologous hydrogenase; (ii) hydrogen production is detected by the H2-sensor of the organism; and, remarkably, (iii) the H2 produced is derived both from fermentative and photosynthetic processes. A manuscript is in preparation. We therefore have created a novel selective means of testing and developing hydrogenases and this system is amenable to directed evolution studies. The heterologous hydrogenase shows some uptake hydrogenase activity as well, and we are currently working to understand if this uptake activity is sufficient to drive photoautotrophic (H2 and CO2) or chemoautotrophic (H2, CO2, and O2) growth of the organism. If so, we may be able to use growth on H2 as an additional selection tool for hydrogenase development.

Publications:

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