IV.E.6 Hydrogen from Water in a Novel Recombinant Oxygen-Tolerant Cyanobacteria System

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Objective

- Develop an O_2-tolerant cyanobacterial system for sustained and continuous light-driven H_2 production from water

Technical Barriers

This project addresses the following technical barrier from the Hydrogen Production section of the Hydrogen, Fuel Cells and Infrastructure Technologies Program Multi-Year Research, Development and Demonstration Plan:

- Z. Continuity of Photoproduction

Technical Targets

Cyanobacteria's hydrogenases are highly O_2-sensitive. Thus, they cannot produce H_2 continuously. This project is performing genetic-engineering studies of cyanobacteria. The outcome of these studies will lead to a much more O_2-tolerant cyanobacteria system, which may be used for continuous photo-hydrogen production. Therefore, this project will facilitate efficient H_2 production from renewable sources and will address the DOE 2010 target for photolytic hydrogen production from water.

Approach

Cyanobacteria have the ability to split water photolytically into O_2 and H_2, but their hydrogenases are highly O_2-sensitive. In contrast, a few anoxygenic photosynthetic bacteria have O_2-tolerant H_2-evolving hydrogenases, but they can not use water as the electron donor. We are using the following two approaches to address this problem.

- Transfer to and express known O_2-tolerant hydrogenases from anoxygenic photosynthetic bacteria in cyanobacteria (Venter Institute and NREL)
  - Determine if the O_2-tolerant hydrogenase can link to the host electronic carrier, ferredoxin
  - Further characterize the O_2-tolerance property of these hydrogenases
  - Transfer and express a known O_2-tolerant hydrogenase from *Thiocapsa roseopersicina* in cyanobacteria (*Synechocystis* and *Synechococcus*)
  - Transfer and express a known O_2-tolerant hydrogenase from *Rubrivivax gelatinosus* in cyanobacteria
- Identify novel O_2-tolerant hydrogenases from Venter Institute's global ocean sampling project, and transfer them into cyanobacteria (Venter Institute)
  - Sample ocean waters, and sequence environmental samples using culture-independent shotgun sequencing approach
  - Construct environmental genomic databases
  - Build Hidden Markov Models (HMMs) and search putative hydrogenase sequences through the databases
  - Retrieve original DNA samples or library for cloning the genes of novel hydrogenases
  - Express the genes and screen for O_2-tolerant hydrogenase
  - Transfer the novel O_2-tolerant hydrogenase into cyanobacteria

Accomplishments

- Transfer to and express foreign O_2-tolerant hydrogenases in cyanobacteria (Venter Institute and NREL)
  - In the past two months, we cloned O_2-tolerant hydrogenase genes *hydS* and *hydL* from *T. roseopersicina* into a cyanobacterial shutter vector. The resulting plasmid, pEX-Tr, was confirmed to carry an intact 3 kb hydS/hydL insert by polymerase chain reactions (PCR), restriction digestion, and complete DNA sequencing. pEX-Tr was transformed into *Synechococcus* PCC7942 using ampicillin as a selection marker. Analysis of these transformants is in process.
  - To obtain active photosynthetic apparatus from *Synechocystis*, we have to isolate cytochrome C6, since this soluble component is normally lost during membrane preparation. We conducted PCR experiments to clone the gene encoding cytochrome C6 (PetJ) for over-expression in *E. coli*. Work is underway to verify its correct gene sequence along with an affinity tag at the C-terminus. Once successful, we will conduct linkage experiments using the photosynthetic apparatus of *Synechocystis* instead.
- Identify novel O_2-tolerant hydrogenases from Venter Institute's global ocean sampling project, and transfer them into cyanobacteria (Venter Institute)
  - As part of the effort to construct an O_2-tolerant cyanobacterial system, we are attempting to identify new O_2-tolerant hydrogenases from marine microbes. To take advantage of the genetic data generated by our Sargasso Sea Water Sampling Project, we used probabilistic modeling methods to search our databases for putative NiFe-hydrogenases. Based on sequence alignments of 89 known hydrogenases, we built 7 HMMs for small subunits and 7 for large subunits. Now, searching is in progress.