DOE Hydrogen Program

Hydrogen from Water in a Novel Recombinant Oxygen-Tolerant Cyanobacterial System

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Project ID
PD039

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Overview

Timeline

- Project start date: 5-01-05
- Project end date: 8-31-2011*
- Percent complete: 90%

Budget

- Total project funding
  - DOE share: $1.62M for JCVI
  - DOE share: $1.26M for NREL
  - JCVI cost-share: $720K
- Funding received for FY10
  - $300K for JCVI
  - $220K for NREL
- Funding for FY11
  - $123K for JCVI
  - $250K for NREL

Barriers

- Barriers addressed
  - Production Barrier AI: Continuity of H_2 production

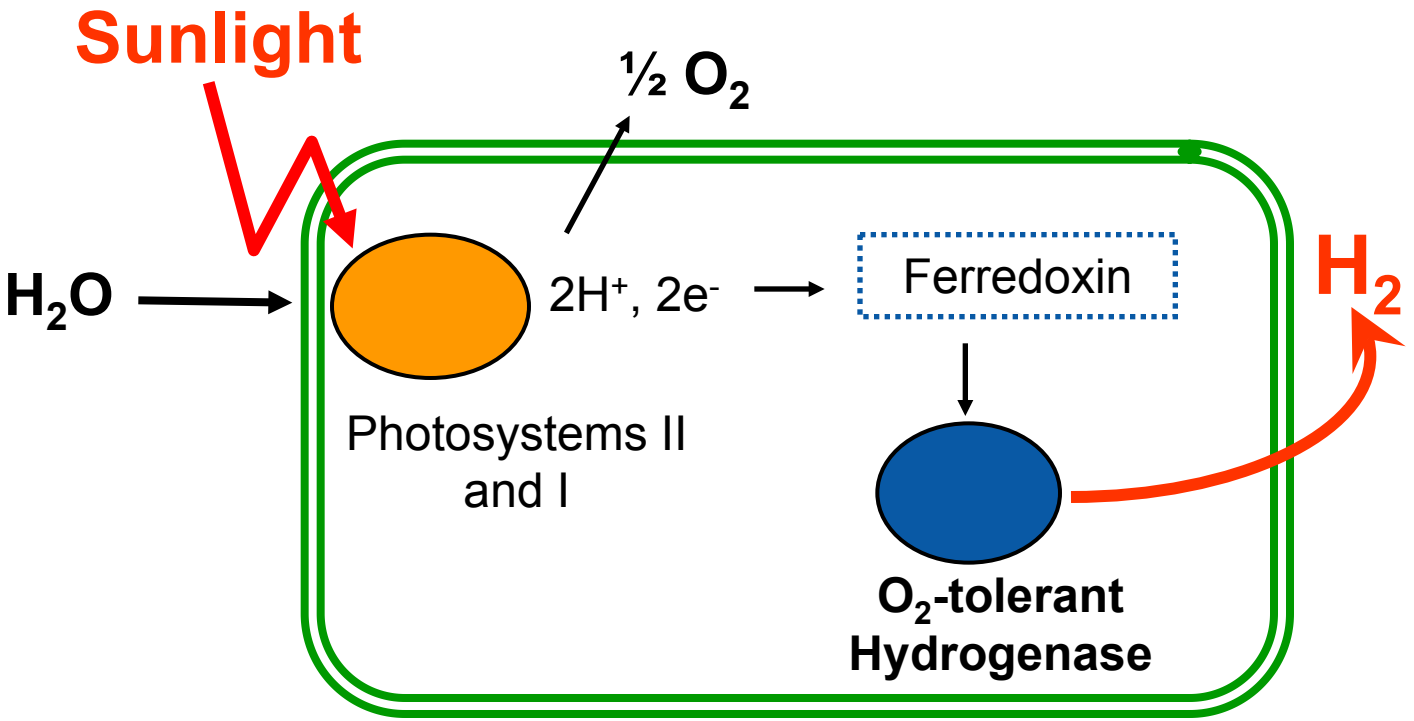
Partners

- J. Craig Venter Institute
- National Renewable Energy Laboratory

*NREL project continuation and direction determined annually by DOE.

Objective-Relevance:

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



Characteristics	2009 Status	2011 Target	2018 Target
Duration of continuous photoproduction	Zero to 30 seconds in air	Produce one cyanobacterial recombinant evolving H_2 through an O_2-tolerant NiFe- hydrogenase	Demonstrate H_2 production in air in a cyanobacterial recombinant

Milestones

Task 1. (JCVI and NREL)

Month/Year	Milestone	% Comp
Sept-09	Purify hydrogenases	JCVI, 100% NREL, 100%
Dec-10	Determine electron mediator requirement	JCVI, 80%
Aug-09	Verify hydrogenase functionality in oxygen	JCVI, 100% NREL, 100%
Sept-11	Construct cyanobacterial hybrid to express an active *Thiocapsa* or *Rubrivivax* hydrogenase	JCVI, 95% NREL, 50%
May-11	Probe functions of two putative hydrogenase maturation genes in assembling the CBS O_2-tolerant hydrogenase	NREL, 50%

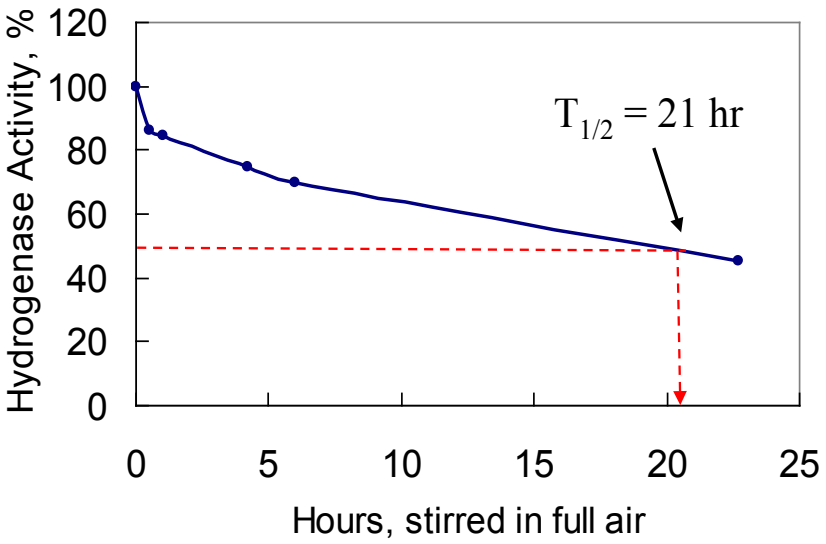
Task 2. (JCVI)

Month/Year	Milestone	% Comp
Sept-09	Identify novel functional hydrogenases from the oceans	JCVI, 100%
Apr-10	Screening for a new O_2-tolerant hydrogenase	JCVI, 100%
Aug-11	Construct a cyanobacterial hybrid to express an active environmental hydrogenase	JCVI, 70%

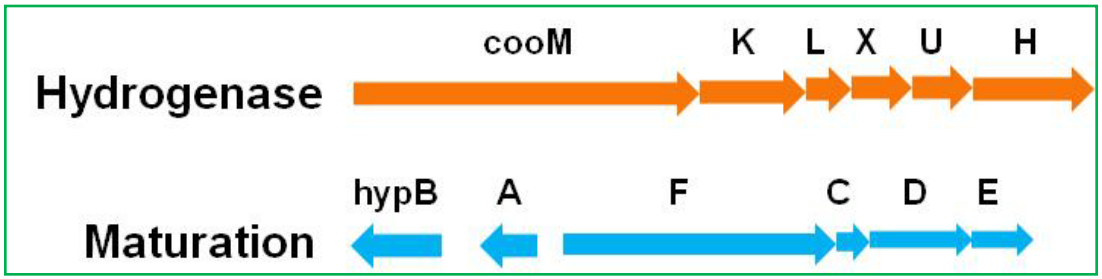
Task 1: NREL Approach

- Transfer an O_2-tolerant NiFe-hydrogenase from the bacterium *Rubrivivax gelatinosus* CBS (hence "CBS", isolated by NREL) into the model cyanobacterium *Synechocystis* sp. PCC 6803

CBS hydrogenase half-life in air: 21 hr



Cloned hydrogenase structural and putative maturation genes

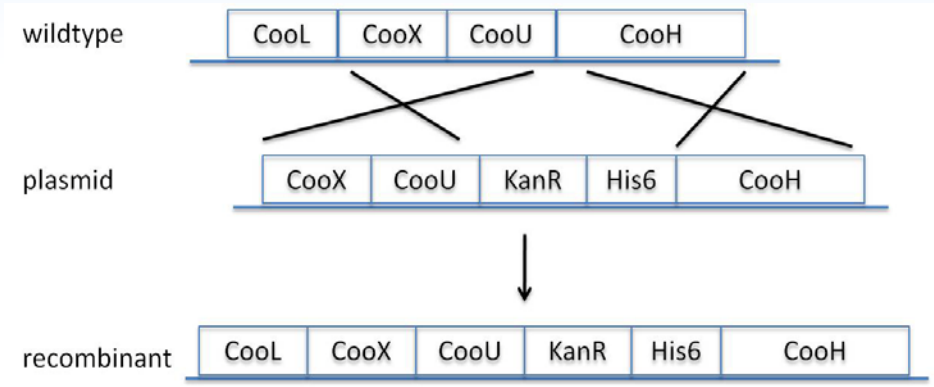


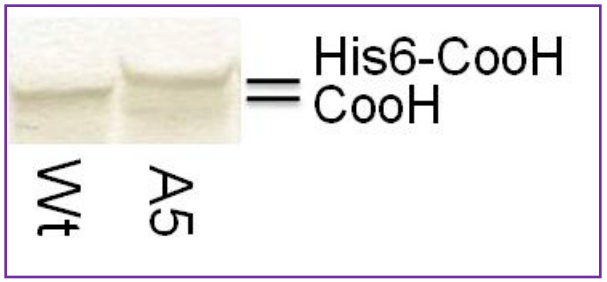
NREL approach is complementary to that of JCVI in harnessing two of Nature's O_2-tolerant hydrogenases and transferring into two model cyanobacteria.

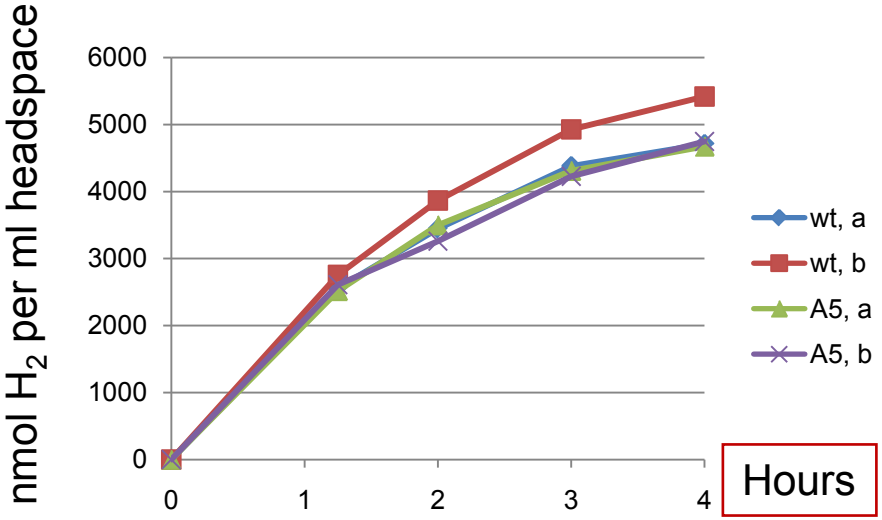
Task 1: NREL Technical Accomplishments

Development of a Genetic System in CBS

- Developed genetic tools to manipulate the genome of CBS, allowing for:
  - Construction of an affinity-tagged CBS hydrogenase for purification.
  - Investigation of functions of the putative *hyp* maturation genes.
- Affinity-tagged CBS hydrogenase was expressed and functional in CBS.





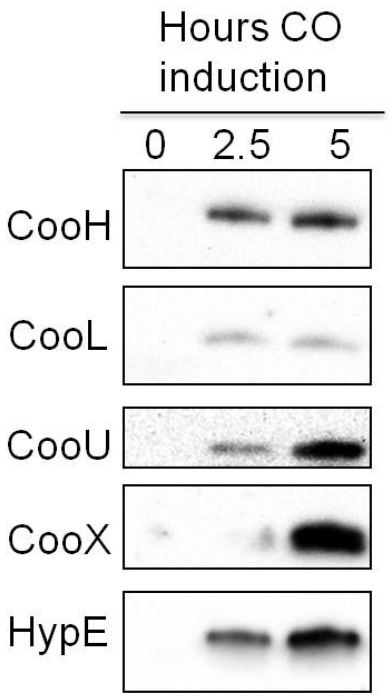


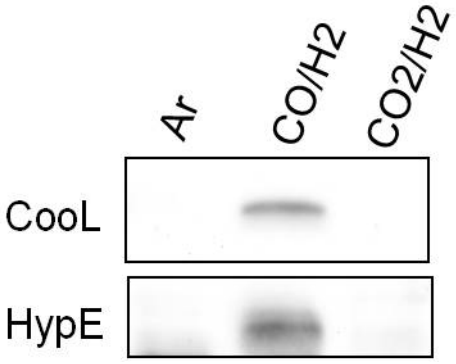
With these tools, the function of the *hyp* genes can be determined to ensure the correct genes are transferred into *Synechocystis*.

Task 1: NREL Technical Accomplishments

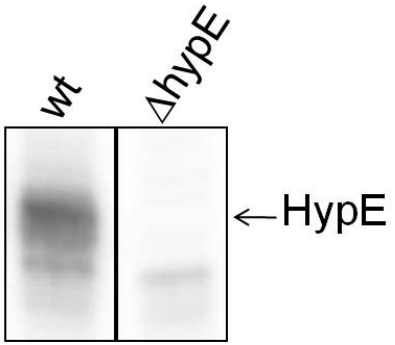
Determine Function of the *hypE* Maturation Gene

- Evidence of HypE as a maturation protein:
  - (1) Co-expression with the CBS hydrogenase subunits.
  - (2) Expression is specific to CO.





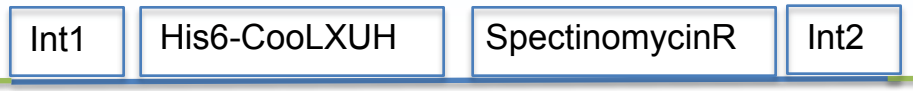
- Surprisingly, loss of *hypE* did not affect hydrogenase activity.
  - Likely due to multiple copies of *hypE* in the genome.
- Work is ongoing to delete *hypF* and *hypCDE* genes.
- Genome sequencing is underway to guide deletions.



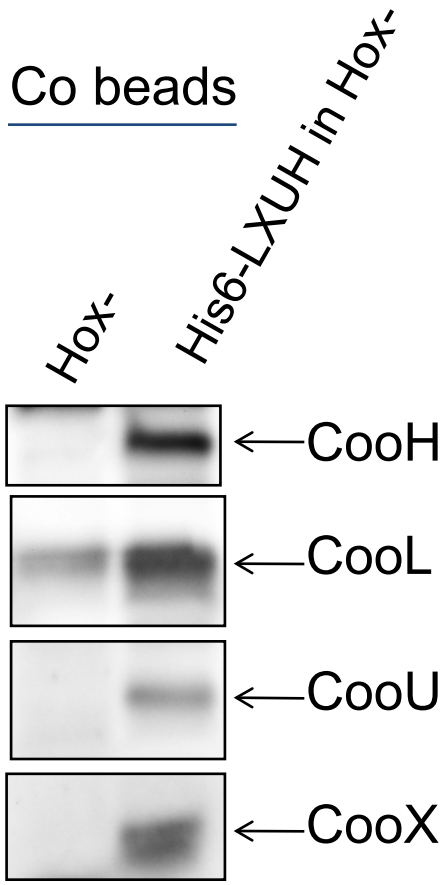
<u>On track</u> to complete Milestone "Probe functions of two putative hydrogenase maturation genes in assembling the CBS O_2-tolerant hydrogenase (5/11).

Task 1: NREL Technical Accomplishments

CBS Hydrogenase Forms a Complex in *Synechocystis*



- In FY10, we expressed CBS hydrogenase (CooLXUH*)* in a hydrogenase-free *Synechocystis* host (Hox-), but expression levels were low.
- In FY11, we transformed codon-optimized *cooLXUH* (N-terminus affinity tag) into *Synechocystis* Hox-.
- Recombinant CBS hydrogenase was purified and shown to contain four subunits (CooLXUH), forming a soluble complex in *Synechocystis*.

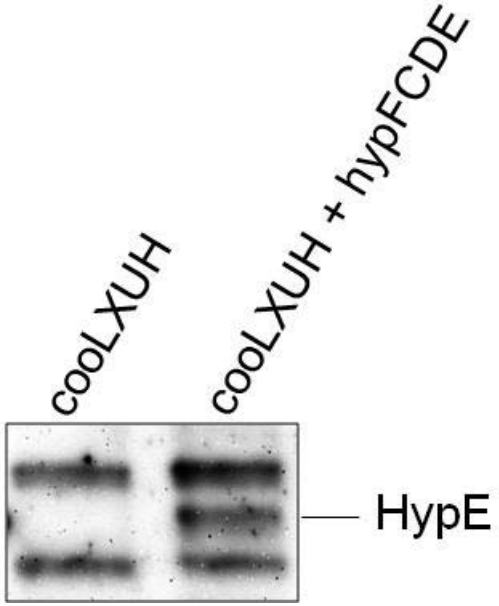


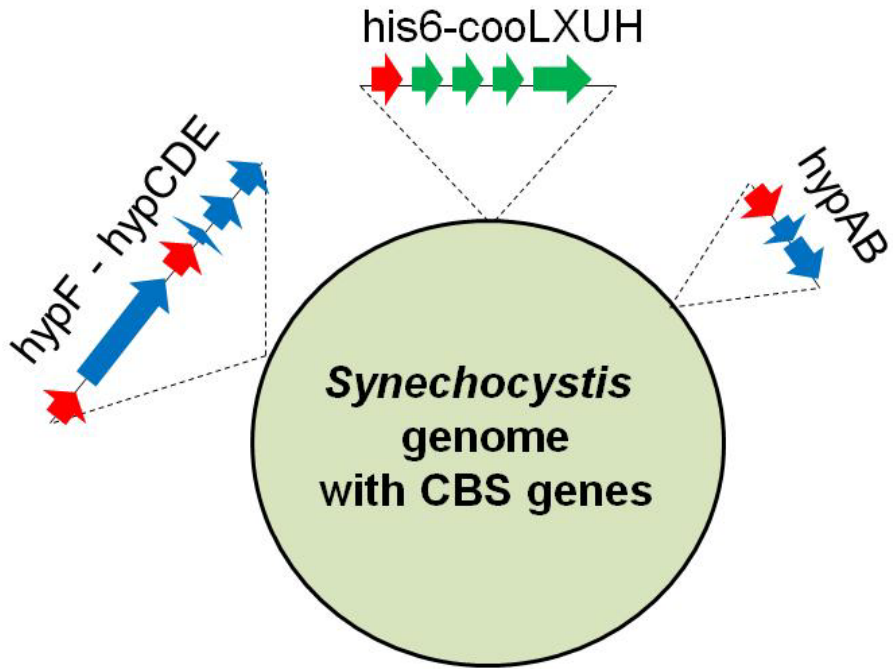
Completed Milestone: Determine compositions of the recombinant hydrogenase (9/10).

Task 1: NREL Technical Accomplishments

Integrate CBS Maturation Genes into *Synechocystis* Genome

- CBS *hypFCDE* genes were integrated into the genome of *Synechocystis.*
- HypE protein was expressed, as shown below.

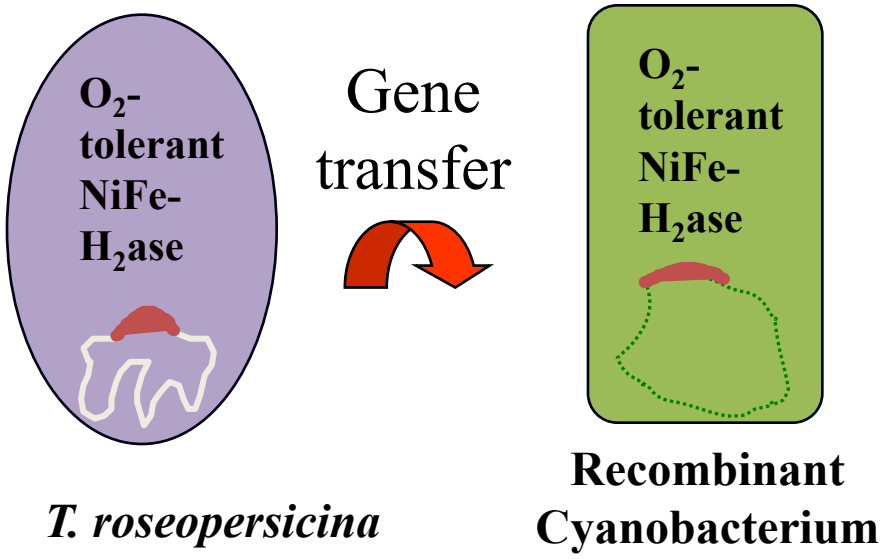


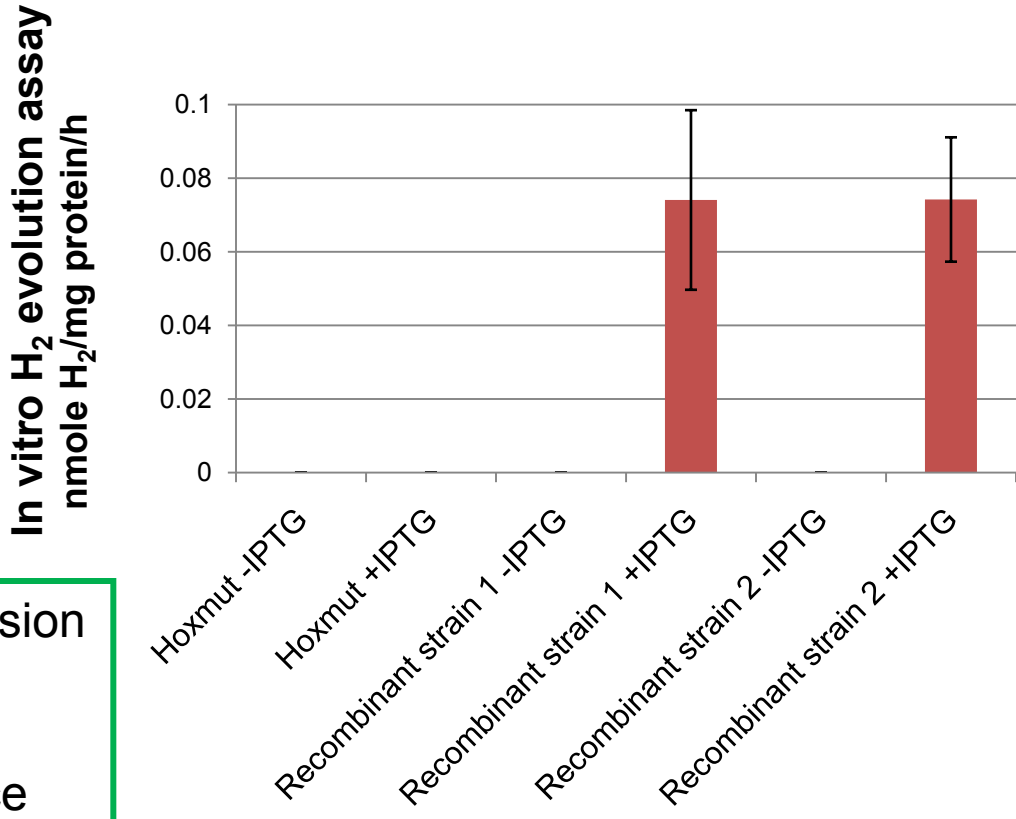


On track to complete Milestone "*Confirm expression of the CBS hydrogenase maturation proteins in recombinant Synechocystis toward producing an active hydrogenase with increased O_2 tolerance*" (9/11).

Task 1: JCVI Technical Accomplishments

Transferring a known O_2-tolerant NiFe-hydrogenase from *T. roseopersicina* into cyanobacterium *Synechococcus sp.* PCC 7942



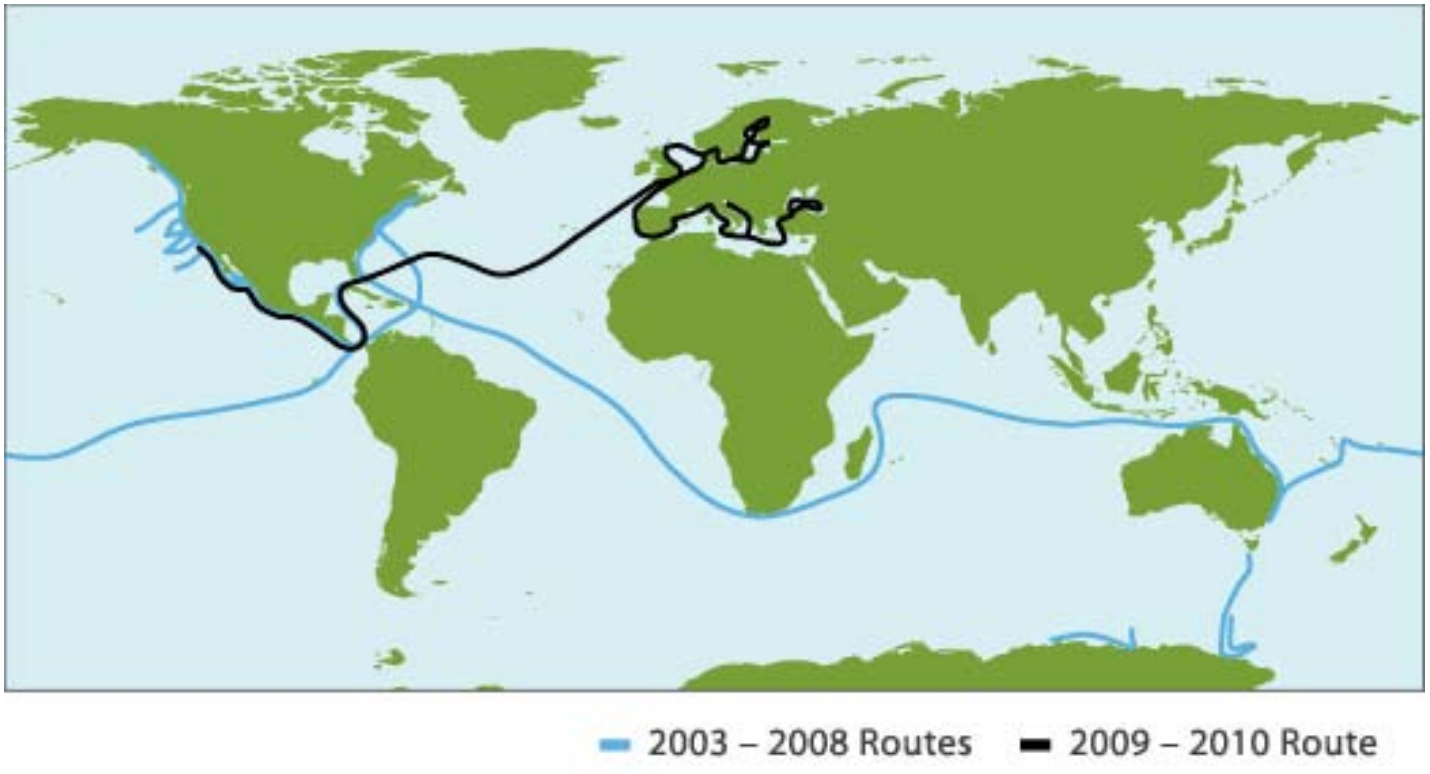


- Effort in FY2011 focused on increasing expression of heterologous hydrogenase – using Envi. Hydrogenase as model.
- We plan to revisit *Thiocapsa* hydrogenase once expression techniques are optimized.
- **<u>On track</u> to complete Milestone "Construct cyanobacterial hybrid to express active Thiocapsa hydrogenase" (8/11).**

- JCVI approach is complementary to that of NREL in harnessing Nature's O_2-tolerant hydrogenases and their transfer into two model cyanobacteria.

Task 2: JCVI Technical Approach

Task 2. Identifying novel O_2-tolerant hydrogenases through metagenomic analysis of marine microbes in the global ocean and transferring the hydrogenases into cyanobacteria



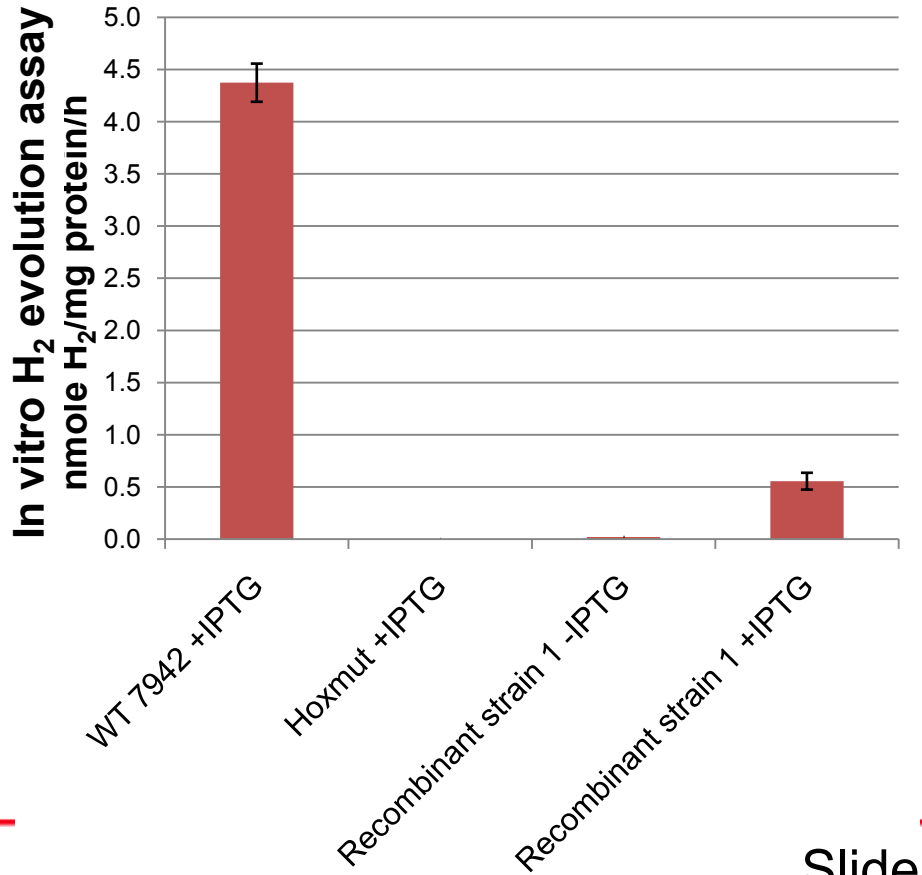
Sorcerer II Expedition: a Global Ocean Sampling Project accomplished by JCVI

- This approach is complementary to two approaches in the Task 1 about harnessing nature's O_2-tolerant hydrogenases and their transfer into cyanobacteria.

Task 2: JCVI Technical Accomplishments

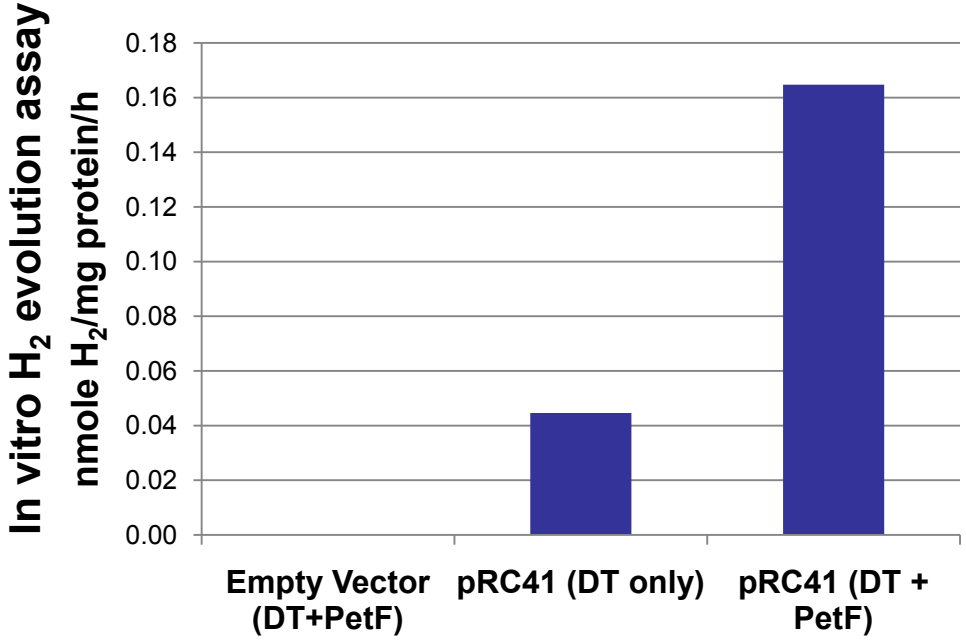
- Previously, we reported transfer of hydrogenase from environmental DNA to various bacterial hosts:
  - *Thiocapsa roseopersicina* (Milestone reached 9/09)
  - *E. coli*
  - Cyanobacteria (*Synechococcus* PCC 7942)

- Purification and biochemical characterization
- Environmental hydrogenase is more thermostable and O_2-tolerant than *Thiocapsa* HynSL.



Task 2: JCVI Technical Accomplishments

- Cyanobacterial ferredoxin (**PetF***) can act as an electron mediator to the environmental hydrogenase in *E. coli* crude extract.



* PetF cloned and purified by NREL and provided to JCVI through our collaboration

- Increasing hydrogenase expression in cyanobacteria may allow for detection of ferredoxin-hydrogenase linkage *in vivo.*

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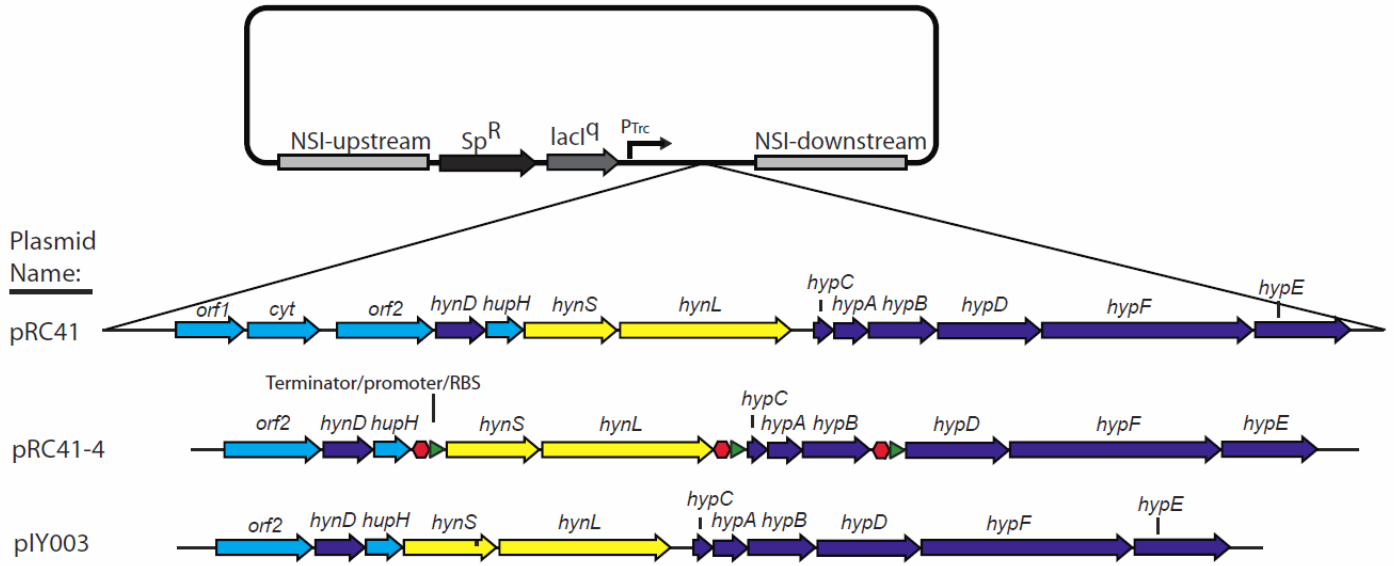
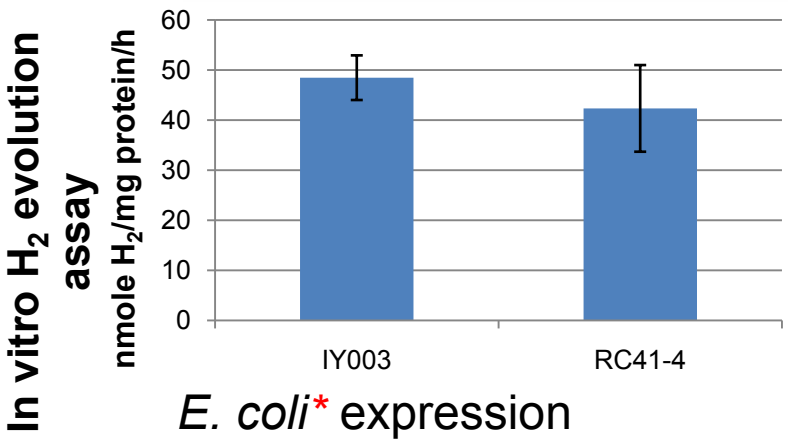
On track to complete Milestone "Determine Electron Mediator Requirement" (12/10)

Task 2: JCVI Technical Accomplishments

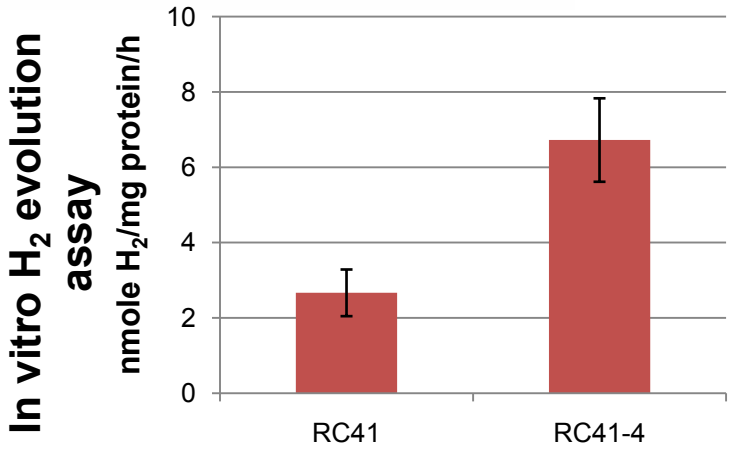
Engineer additional promoters into environmental hydrogenase gene cluster to increase hydrogenase activity.





*E. coli** expression

(*E. coli strain developed by NREL)



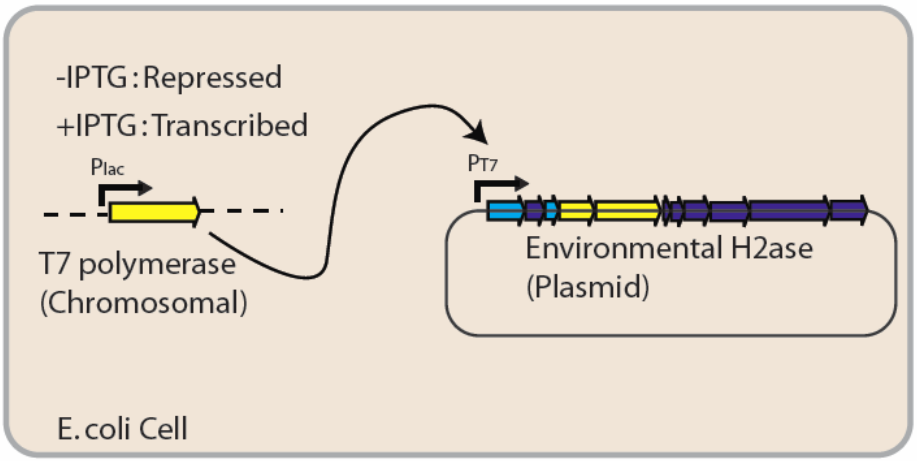
Cyanobacteria expression

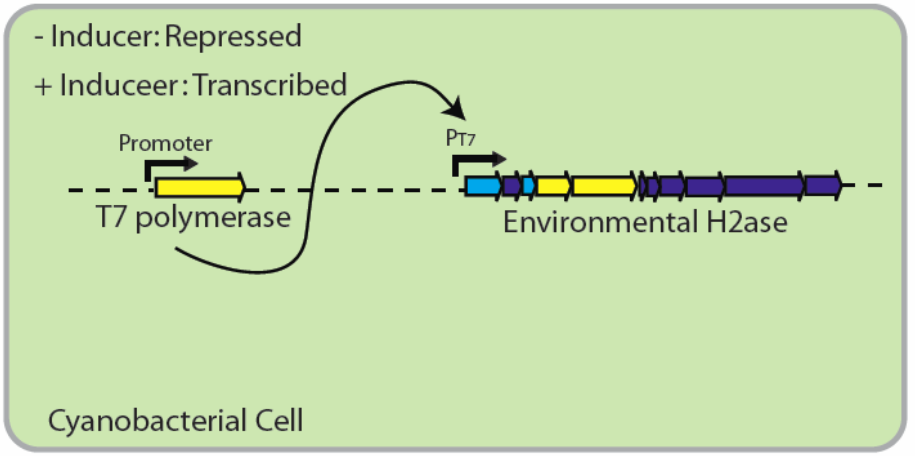
On track to complete Milestone "Construct a cyanobacterial hybrid to express an active environmental hydrogenase" (8/11).

Task 2: JCVI Technical Accomplishments

Increasing hydrogenase activity – Continued:

- Engineer T7 polymerase strategy for hydrogenase expression.





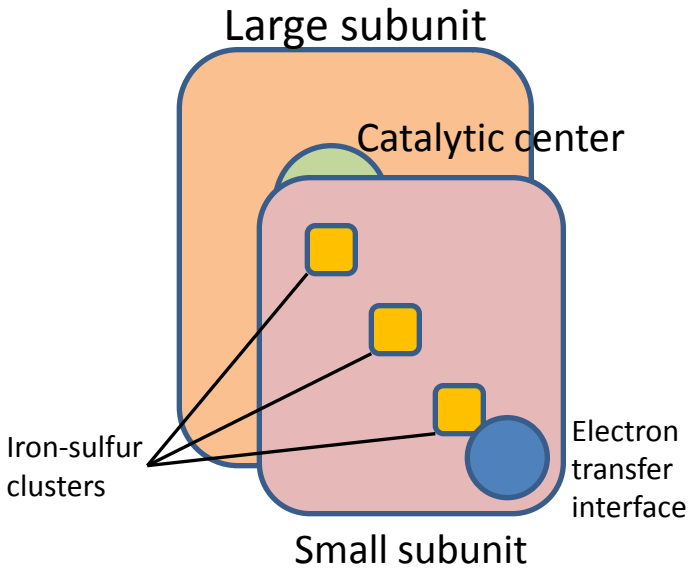
Advantages of T7 polymerase:

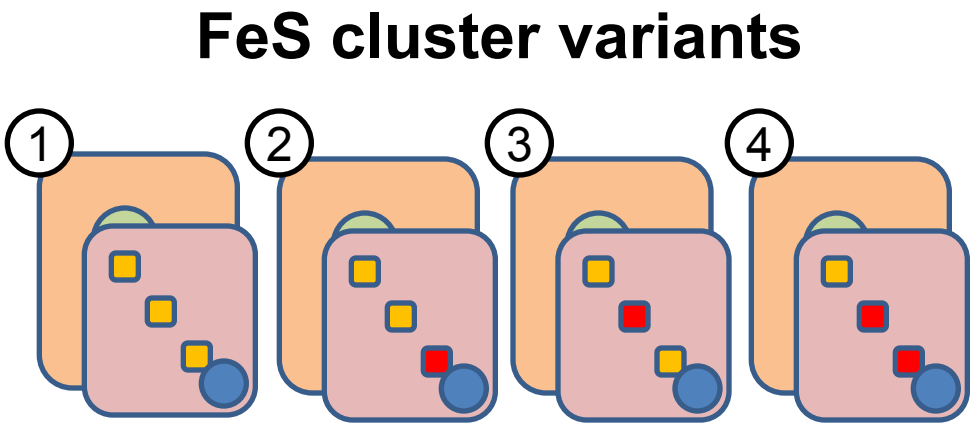
- Tighter control of transcription
- Better transcriptional processivity, may function better with long transcripts.

On track to complete Milestone "Construct a cyanobacterial hybrid to express an active environmental hydrogenase" (8/11).

Task 2: JCVI Technical Accomplishments

Point mutants alter electrochemistry of the hydrogenase small subunit "molecular wire"





Tests the potential to further modify the environmental hydrogenase to favor H_2 production *in vivo* using PetF as an electron mediator.

On track to complete Milestone "Determine Electron Mediator Requirement" (12/10).

Collaborations

JCVI

- Vanderbilt University
  - Expressing O_2-tolerant hydrogenases in cyanobacteria

NREL

- Qingdao Institute of Bioenergy and Bioprocess Technology (China)
  - Purify native CBS hydrogenase for characterization
- Michigan State University
  - Sequence and annotate CBS genome

Proposed Future Work

JCVI

- Continue work to increase O_2-tolerant hydrogenase expression in cyanobacteria via optimization of transcriptional regulation (FY11 and 12)
- Measure expression of accessory genes in optimized system to determine contribution to increased activity (FY11 and 12)
- Improve electron transfer between ferredoxin and hydrogenase small subunit (FY11 and 12)
- Demonstrate linkage between environmental hydrogenase and photosystem in cyanobacterial system (FY11 and 12)

NREL

- Continue to probe hydrogenase maturation machineries in CBS, taking advantage of the CBS genetic tools we developed (FY11 and 12).
- Express additional CBS hydrogenase maturation genes and measure hydrogenase activity in *Synechocystis* host (FY11 and 12).
- Improve linkage of CBS hydrogenase to the *Synechocystis* photosynthetic pathways to enhance photolytic H_2 production (FY12).
- Purification of the affinity-tagged CBS hydrogenase to test its functionality in O_2 (FY12)

Summary

JCVI

- Purified O_2-tolerant NiFe hydrogenase from *Thiocapsa*, performed O_2-tolerance and thermostability tests, and successfully expressed functioning hydrogenase in cyanobacteria.
- Identified a novel, O_2-tolerant NiFe hydrogenase from environmental DNA, expressed functioning hydrogenase in several hosts including cyanobacteria, and characterized for O_2-tolerance and thermostability.
- Increased expression of the environmentally-derived hydrogenase in cyanobacteria by modifying the transcriptional strategy.
- Verified linkage between the cyanobacterial ferredoxin and the environmental hydrogenase.

NREL

- Developed genetic tools in CBS to manipulate its genome at ease.
- Produced an affinity-tagged CBS hydrogenase for purification and characterization.
- Preliminary evidence suggests that *hypE* is a hydrogenase maturation gene.
- Expressed four CBS hydrogenase subunits (CooLXUH) that form a soluble complex in *Synechocystis.*
- Integrated four putative CBS maturation genes in the *Synechocystis* genome, with expression of HypE confirmed by western blot.