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

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National Renewable Energy Laboratory

Project ID
PD039

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Overview

Timeline

- Project start date: 5-01-05
- Project end date: 1-30-2014*
- Percent complete: 80%

Budget

- Total project funding
  - DOE share: $2.019M for JCVI
  - DOE share: $1.51M for NREL
  - JCVI cost-share: $820K
- Funding received for FY11
  - $123K for JCVI
  - $250K for NREL
- Planned funding for FY12
  - $150K for JCVI
  - $350K for NREL

Barriers

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

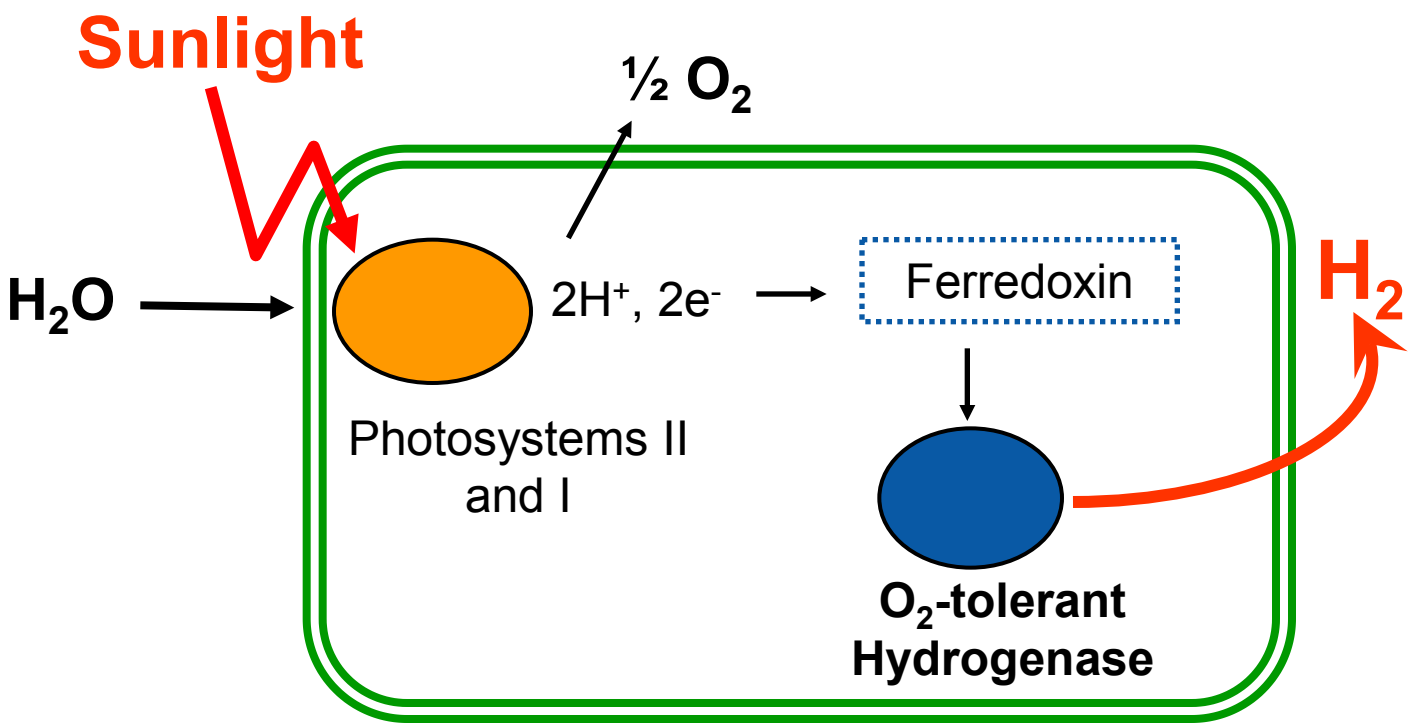
Partners

- 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
Dec-10	Determine electron mediator requirement	JCVI,100%
May-11	Probe functions of two putative hydrogenase maturation genes in assembling the CBS O_2-tolerant hydrogenase	NREL, 100%
Sept-11	Confirm expression of the CBS hydrogenase maturation proteins in recombinant *Synechocystis*	NREL, 100%
May-12	Identify two additional hydrogenase maturation genes via quantitative PCR and the analysis of CBS genome	NREL, 100%
Sept-12	Generate a *Synechocystis* recombinant evolving H_2 via the CBS hydrogenase	NREL, 80%

Task 2. (JCVI)

Month/ Year	Milestone	% Comp
Aug-11	Construct a cyanobacterial hybrid to express an active environmental hydrogenase	JCVI, 100%
Apr-13	Increase activity of HynSL hydrogenase in cyanobacteria to give 100-fold increase in specific activity.	JCVI, 5%
Nov-13	Improve hydrogenase-ferredoxin (Fd) electron transfer to enable 25-fold better Fd docking to the hydrogenase.	JCVI, 5%
Jan-14	Measure light-dependent H_2 production in modified strains.	JCVI, 5%

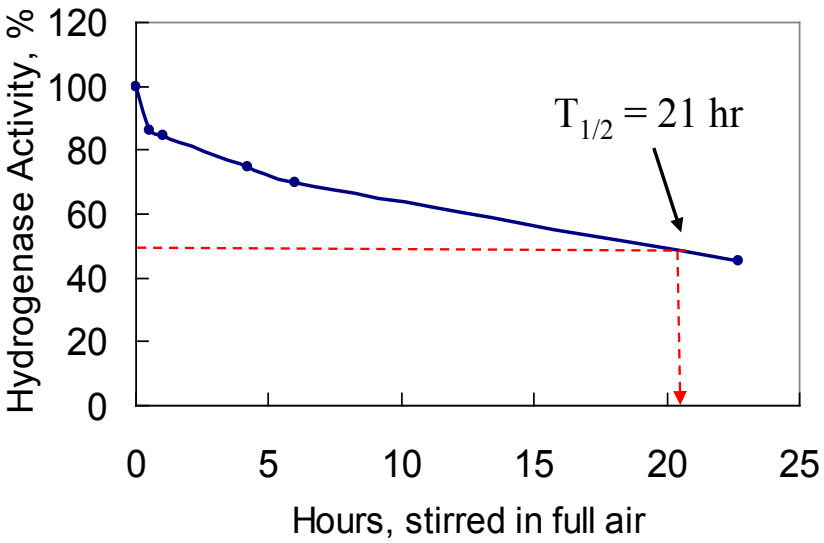
Go/No Go Decision: Jan-2013

Demonstrate 5x increase hydrogenase activity from environmental H2ase in cyanobacteria as measured by in vitro H2 evolution assay.

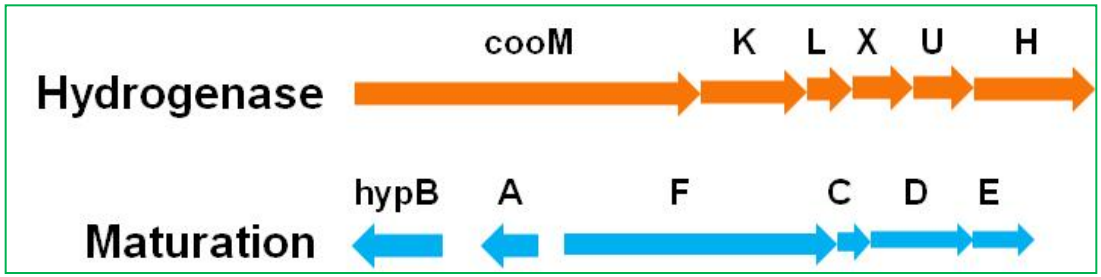
Task 1: NREL Approach

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

CBS hydrogenase half-life in air: 21 hr



Cloned hydrogenase structural and putative maturation genes

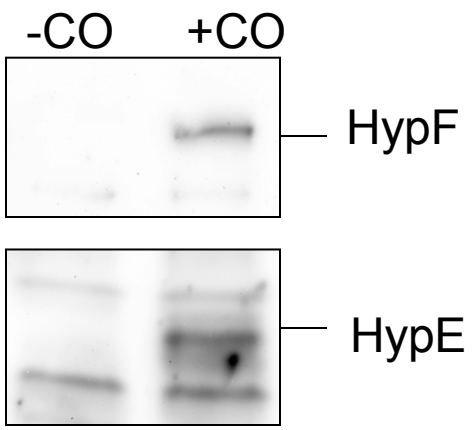


NREL approach is complementary to that of JCVI in harnessing two of Nature's O_2-tolerant hydrogenases and transferring into two model cyanobacteria.

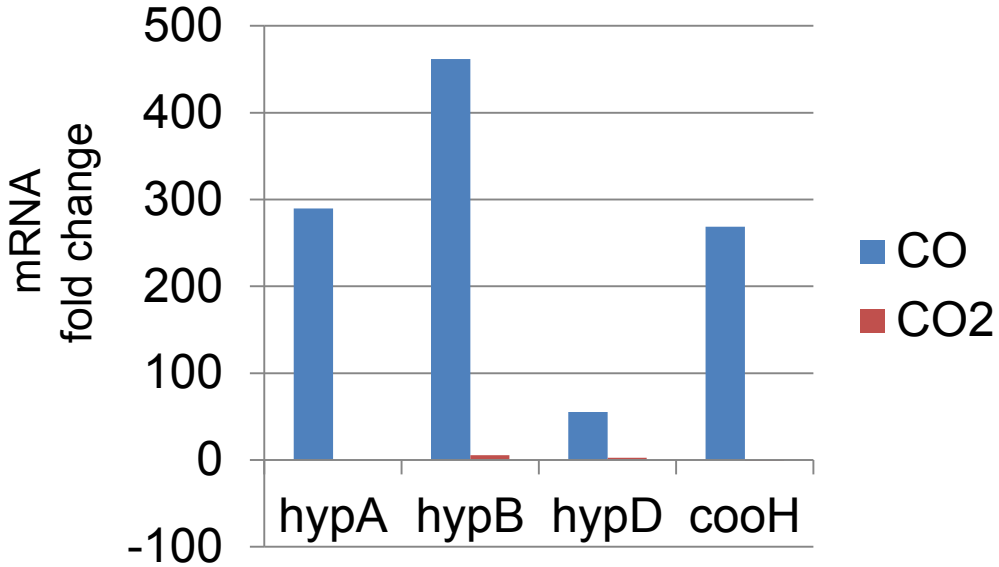
Task 1: NREL Technical Accomplishments

Determined Function of the *hypABDF* Maturation Genes

- Protein immunoblot verified *hypF* as a maturation gene
  - Protein expression is specific to CO.



- Quantitative RT-PCR verified *hypABD* as maturation genes
  - Gene expression is specific to CO

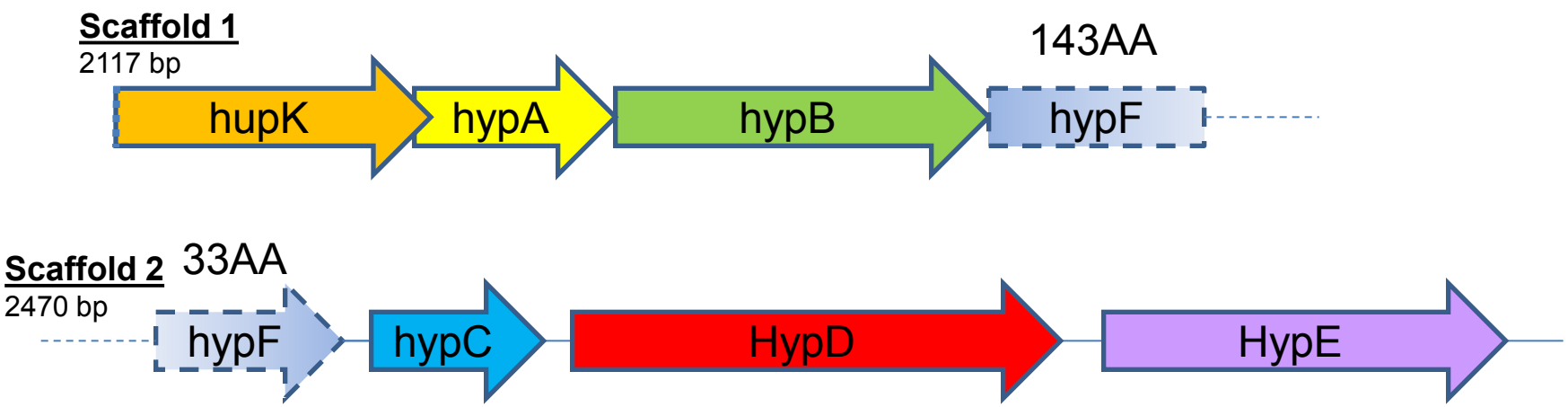


- Completed Milestone "Probe functions of two putative hydrogenase maturation genes in assembling the CBS O_2-tolerant hydrogenase (5/11).
- Completed Milestone "Identify two additional hydrogenase maturation genes via quantitative PCR and analysis of CBS genome (5/12).

Task 1: NREL Technical Accomplishments

Identified Additional Hydrogenase Maturation Genes

- NREL collaborates with Michigan State U. (leveraging Office of Science funding) and Pacific Biosciences (free service) in sequencing and annotating CBS genome.
  - 5 MB genome size, 3,582 genes with predicted function.
- Analysis of CBS genome revealed a new set of *hyp* genes.



- Via PCR we filled the gap in the *hypF* gene and confirmed these maturation genes are in a single operon.

- **Completed Milestone "Identify two additional hydrogenase maturation genes via quantitative PCR and the analysis of CBS genome (5/12).**

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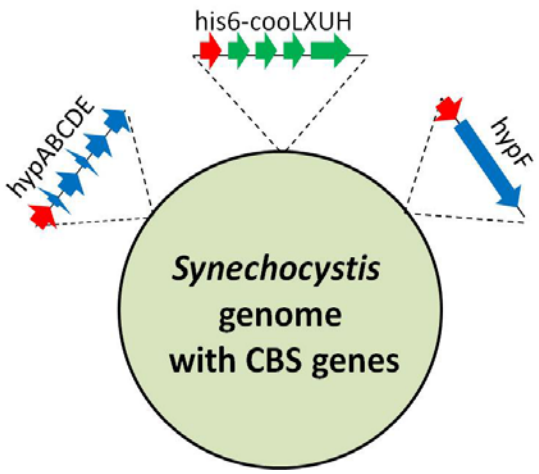
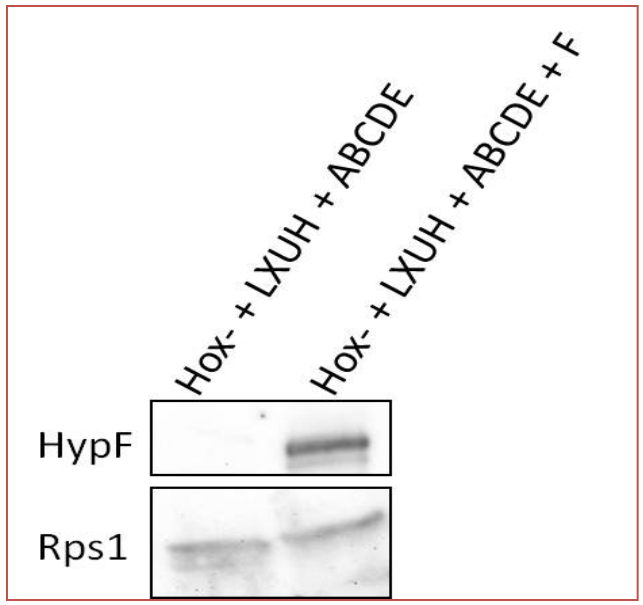
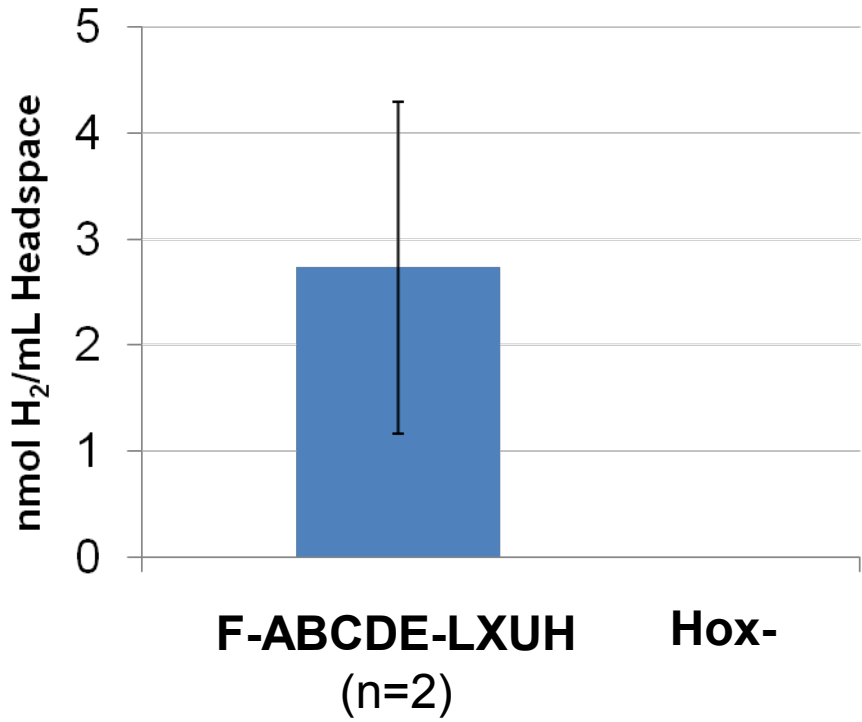
Task 1: NREL Technical Accomplishments

Synechocystis Recombinant #1 with CBS Hydrogenase Activity

- **Ten** CBS genes were transformed into a *Synechocystis* recombinant in which its native hydrogenase was inactivated (Hox-).
- The recombinant expressed HypF protein and displayed CBS hydrogenase activity.







- **Completed** Milestone "*Confirm expression of the CBS hydrogenase maturation proteins in recombinant Synechocystis*" (9/11).
- **On track** to complete Milestone "Generate a *Synechocystis* recombinant evolving H_2 via the CBS hydrogenase" (9/12).

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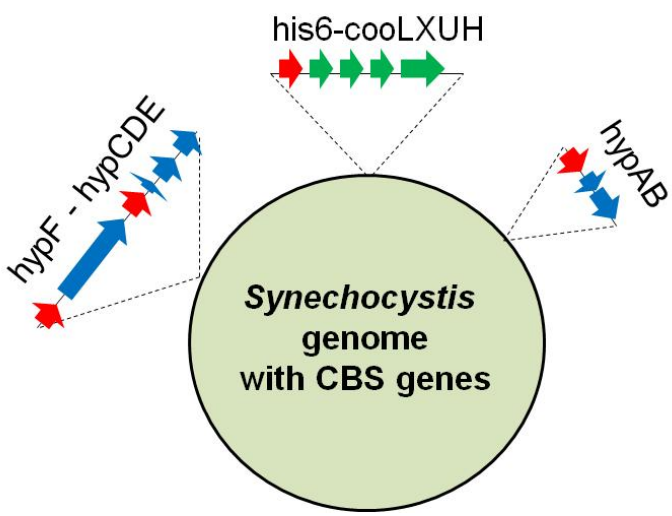
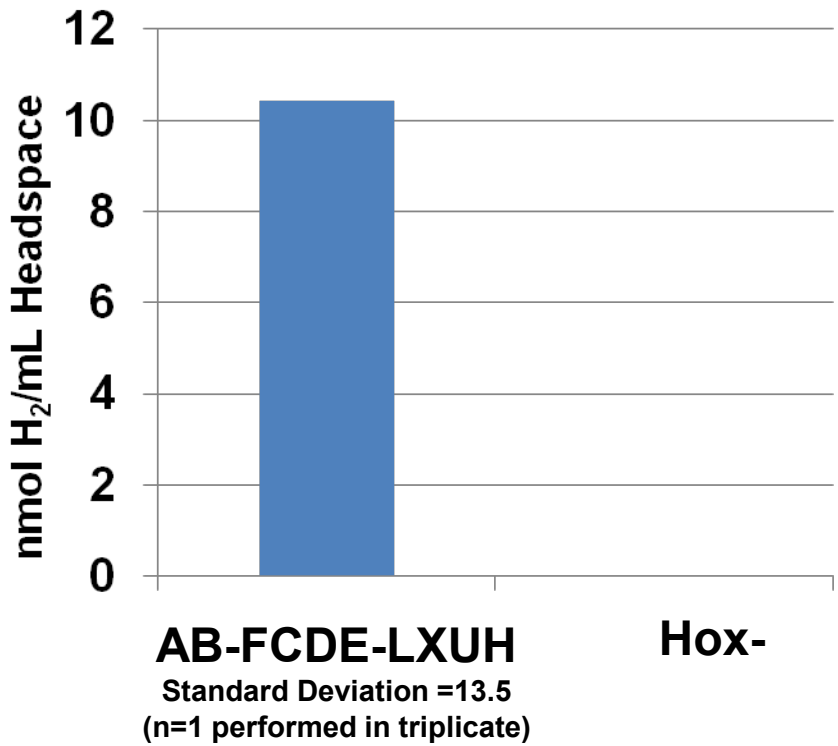
Task 1: NREL Technical Accomplishments

Synechocystis Recombinant #2 with CBS Hydrogenase Activity

- Generated a second *Synechocystis* recombinant displaying CBS hydrogenase activity, with different promoters arrangement.
- Promoter engineering is ongoing to improve activity further.

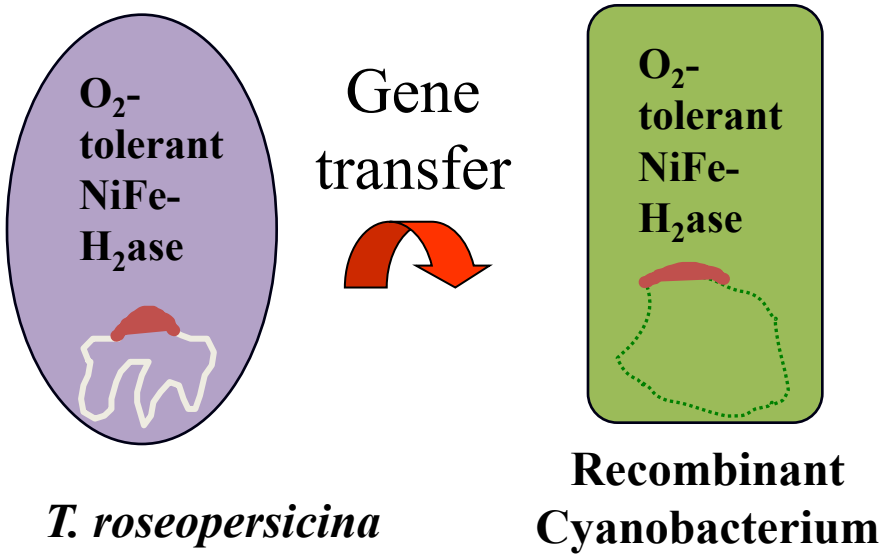


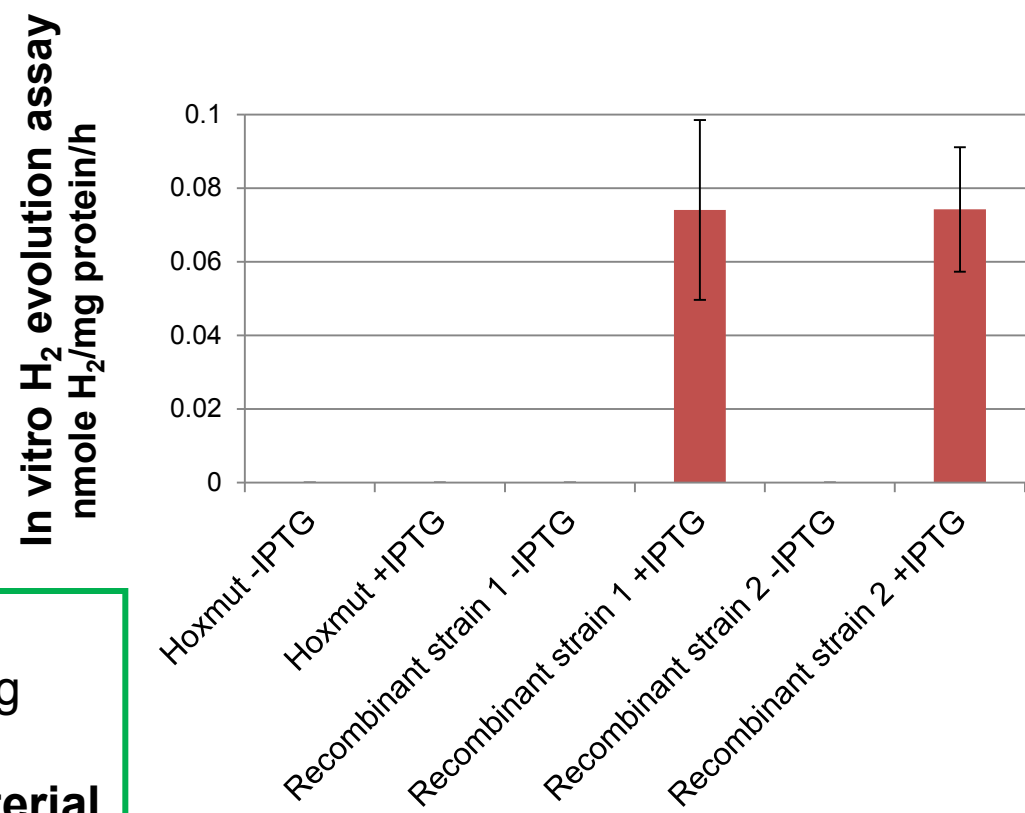


On track to complete Milestone "Generate a *Synechocystis* recombinant evolving H_2 via the CBS hydrogenase" (9/12).

Task 1: JCVI Technical Accomplishments

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



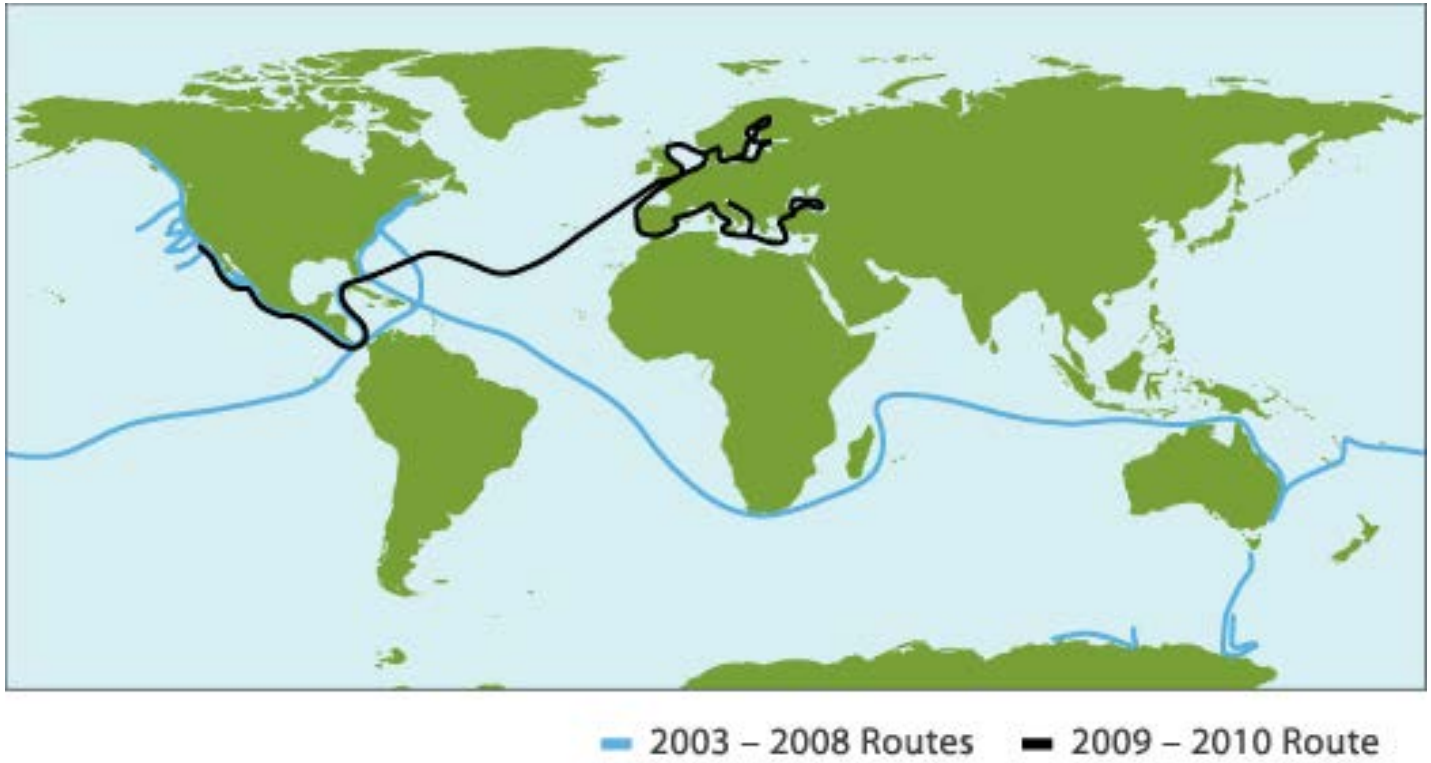


- Effort in FY2011-12 focused on increasing expression of heterologous hydrogenase – using Envi. Hydrogenase as model.
- **Completed milestone "Construct cyanobacterial hybrid to express active *Thiocapsa* hydrogenase" (8/11).**

- 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



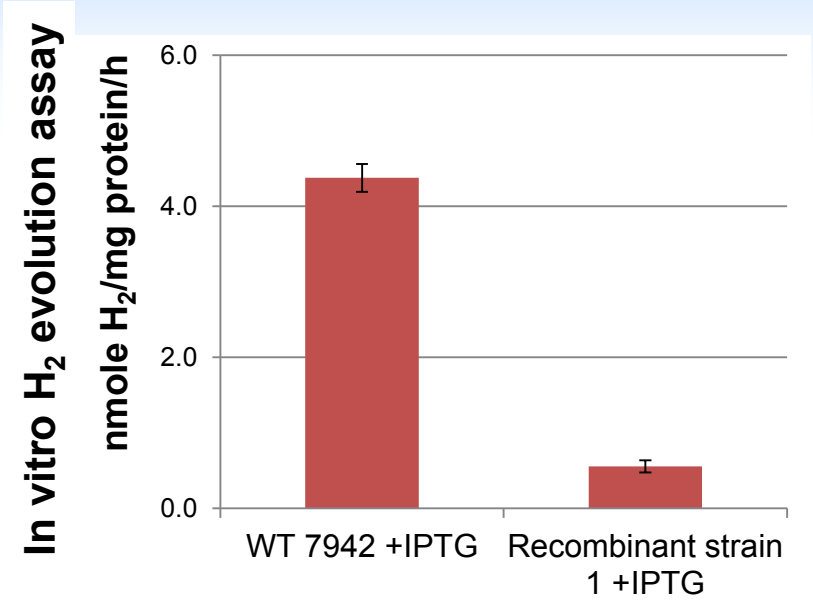
Sorcerer II Expedition: a Global Ocean Sampling Project accomplished by JCVI

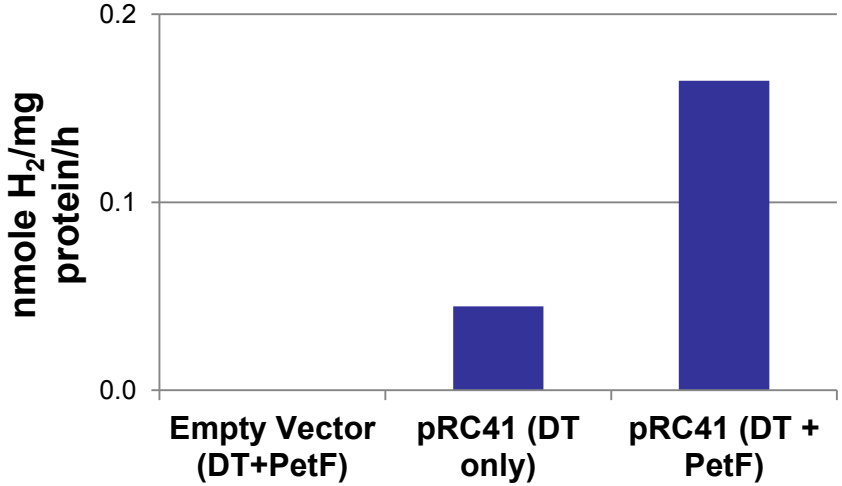
- This approach is complementary to two approaches in the Task 1 about harnessing nature's O_2-tolerant hydrogenases and their transfer into cyanobacteria.

Task 2: JCVI Technical Accomplishments

- Previously, we reported transfer of hydrogenase from environmental DNA to *Synechococcus* PCC 7942 (Milestone reached 4/10)
- Environmental hydrogenase is more thermostable and O_2-tolerant than *Thiocapsa* HynSL.
- 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

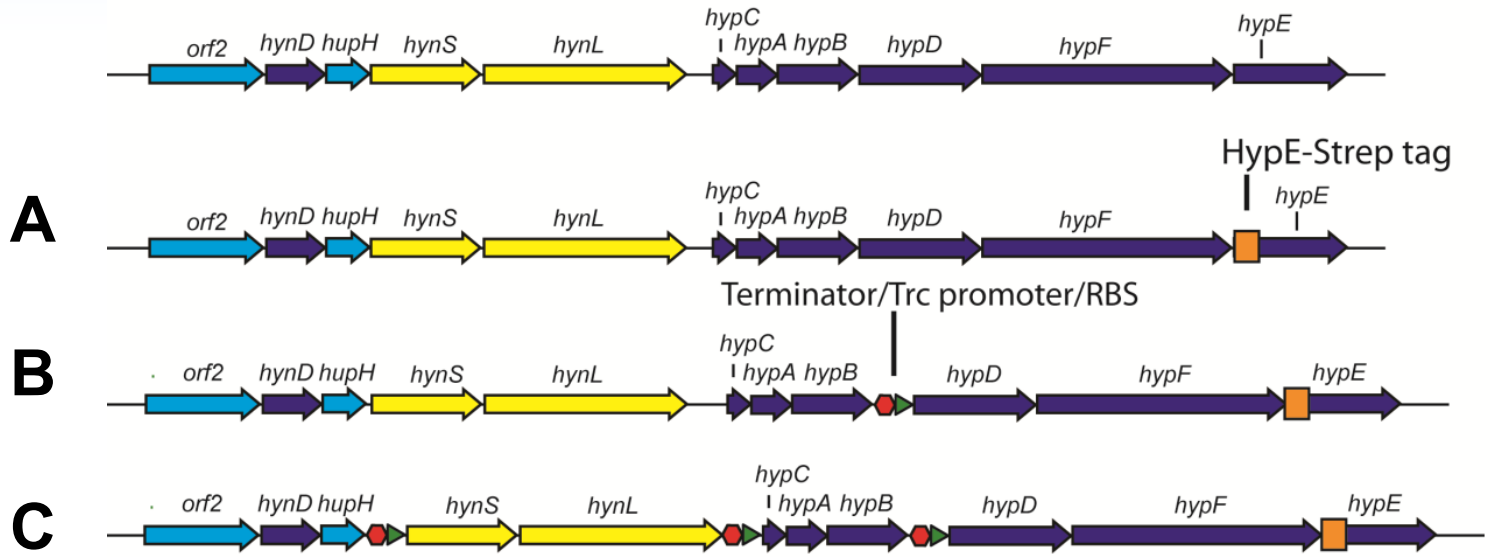


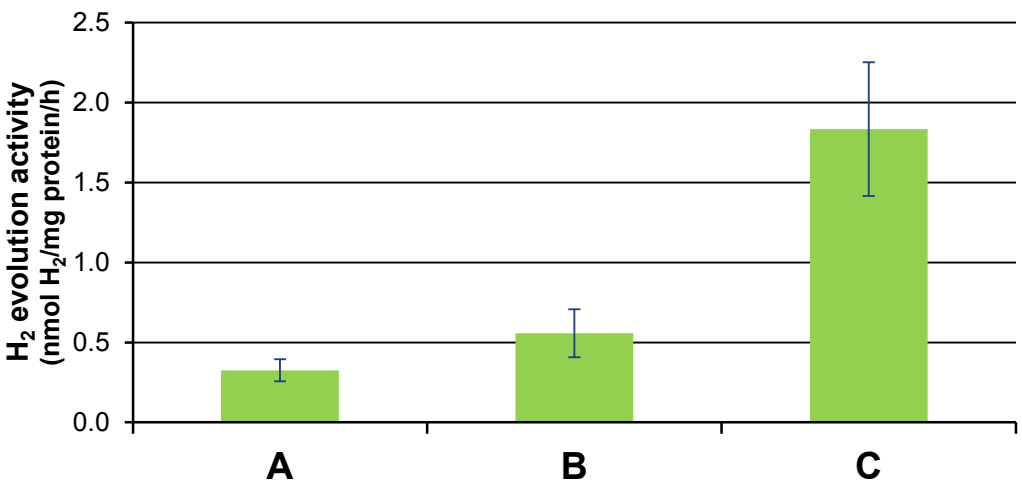


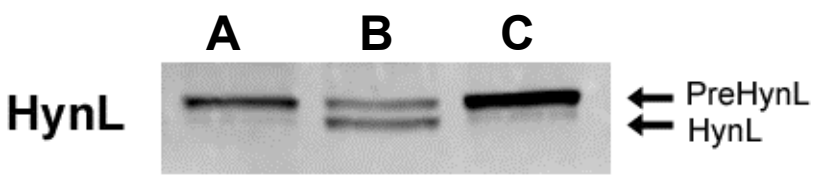
Completed Milestone "Determine Electron Mediator Requirement" (12/10)

Task 2: JCVI Technical Accomplishments

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







<u>On track</u> to complete Milestone "Increase activity of HynSL hydrogenase in cyanobacteria to give 100-fold increase in specific activity." (4/13).

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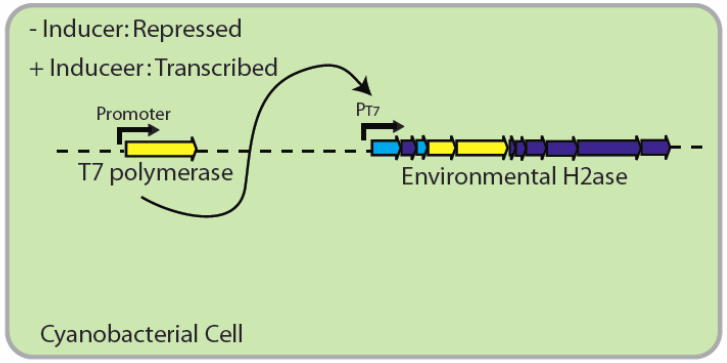
Task 2: JCVI Technical Accomplishments

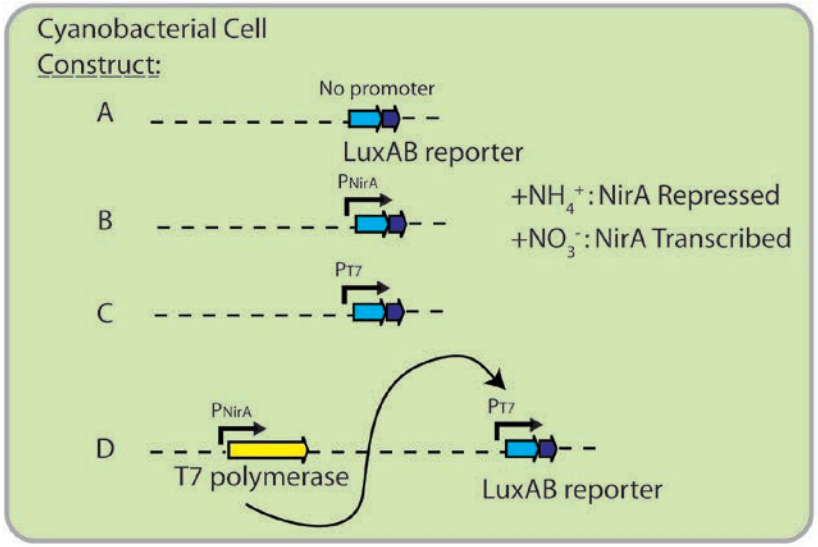
Increasing hydrogenase activity – Continued:

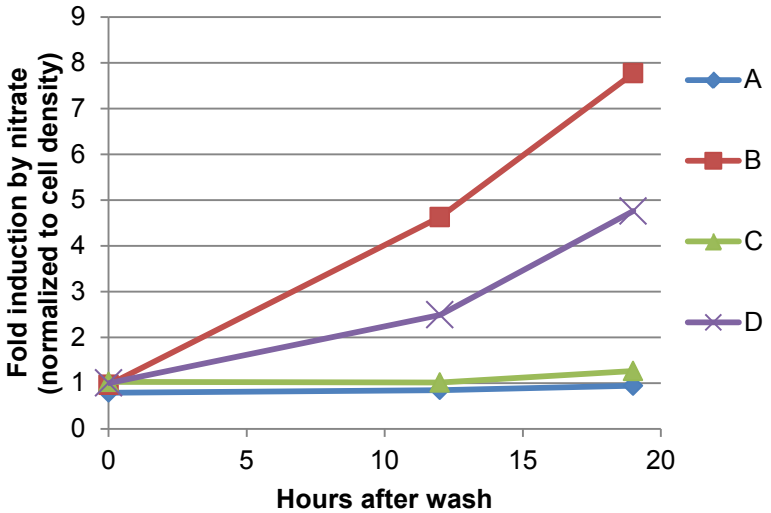
- Engineer T7 polymerase strategy for hydrogenase expression.



Advantages of T7 polymerase:

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





On track to complete Milestone "Increase activity of HynSL hydrogenase in cyanobacteria to give 100-fold increase in specific activity." (4/13).

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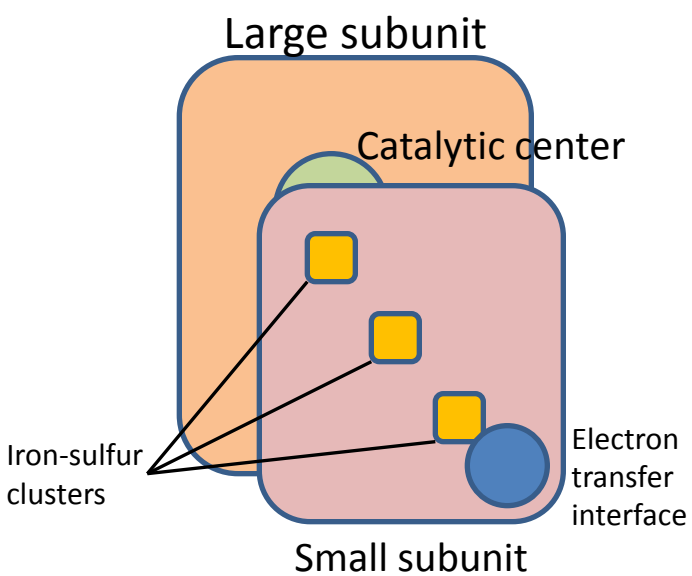
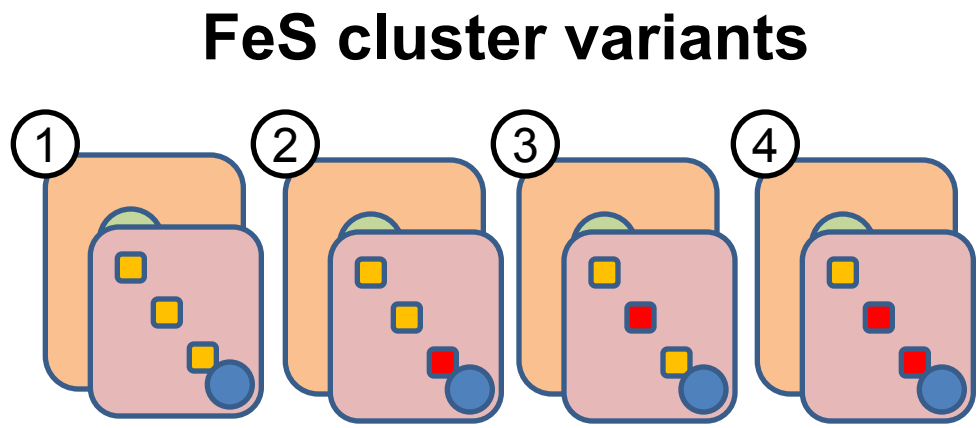
Task 2: JCVI Technical Accomplishments

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

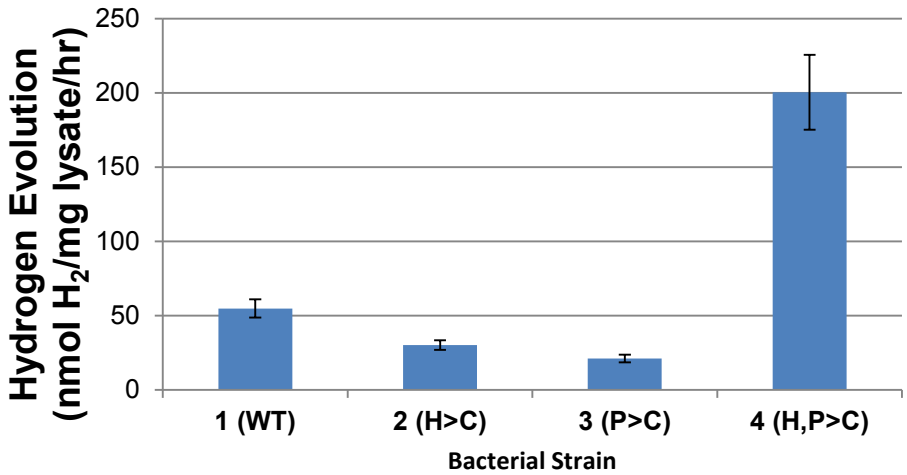


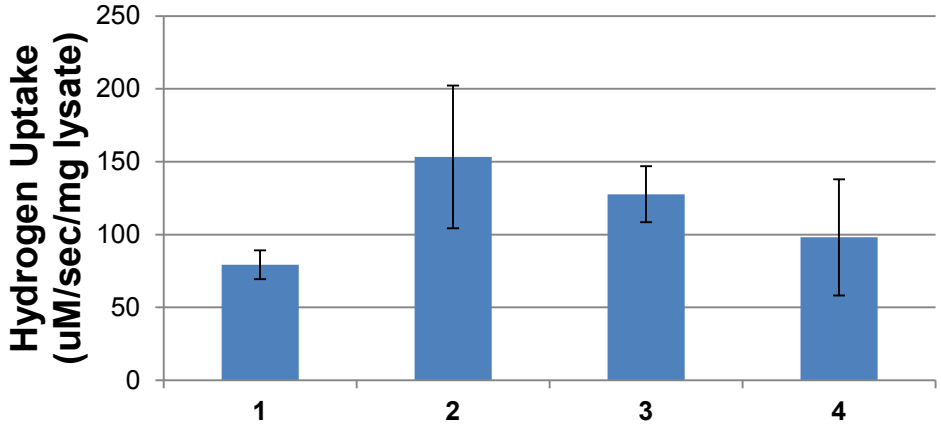


Tests the potential to further modify the environmental hydrogenase to favor H_2 production *in vivo* using PetF as an electron mediator.

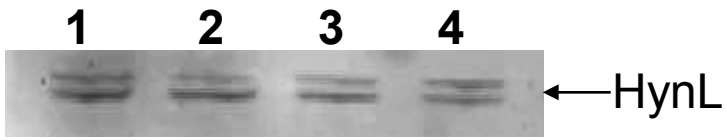
Task 2: JCVI Technical Accomplishments

Double mutant has increased evolution activity relative to uptake





Western blot of HynL shows equivalent expression in all samples



<u>On track</u> to complete Milestone "Improve hydrogenase-ferredoxin (Fd) electron transfer to enable 25-fold better Fd docking to the hydrogenase." (11/13).

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Collaborations

JCVI

- Vanderbilt University
  - Expressing O_2-tolerant hydrogenases in cyanobacteria

NREL

- Michigan State University
  - Annotate CBS genome
  - Leverage funding from DOE Office of Science
- Pacific Biosciences
  - Provide **free** CBS genome sequencing

Proposed Future Work

JCVI

- Continue work to increase O_2-tolerant hydrogenase expression 100-fold in cyanobacteria via optimization of transcriptional regulation of hydrogenase and accessory genes and by engineering FeS cluster mutations (FY12 and 13)
- Improve electron transfer between ferredoxin and hydrogenase small subunit by mutational analysis and constructing ferredoxin-hydrogenase fusion proteins (FY12 and 13)
- Link environmental-hydrogenase to S. elongatus photosynthetic pathways (FY13).

NREL

- Probe roles of a set of newly discovered hydrogenase maturation genes in CBS identified from the sequenced genome (FY12).
- Manipulate promoters to increase CBS hydrogenase activity in the *Synechocystis* recombinants (FY12 & FY13).
- Link CBS hydrogenase to the *Synechocystis* photosynthetic pathways to enhance photolytic H_2 production (FY13).

Summary

JCVI

- Increased expression of the environmentally-derived hydrogenase in cyanobacteria by modifying the transcriptional strategy.
- Developed a novel T7 polymerase strategy for expression of hydrogenase.
- Identified mutations leading to increased hydrogenase activity in the direction of hydrogen evolution.

NREL

- CBS genome sequencing and annotation is near complete, at no cost to DOE.
- A new set of hydrogenase maturation genes is identified through the above genome sequencing effort.
- Confirmed roles of four hydrogenase maturation genes involved in the assembly of the CBS O_2-tolerant hydrogenase.
- Generated two *Synechocystis* recombinants which displayed active CBS hydrogenase activity, albeit improvement is needed.