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

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Project ID
PD039

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J. Craig Venter
INSTITUTE

Overview

Timeline

- Project start date: 5-01-05
- Project end date: 8-31-2011
- Percent complete: 80%

Budget

- Total project funding
  - DOE share: $1.62M for JCVI
  - DOE share: $1.26M for NREL
  - JCVI cost-share: $720K
- Funding received for FY09
  - $100K for JCVI
  - $220K for NREL
- Funding for FY10
  - $300K for JCVI
  - $86K for NREL

Barriers

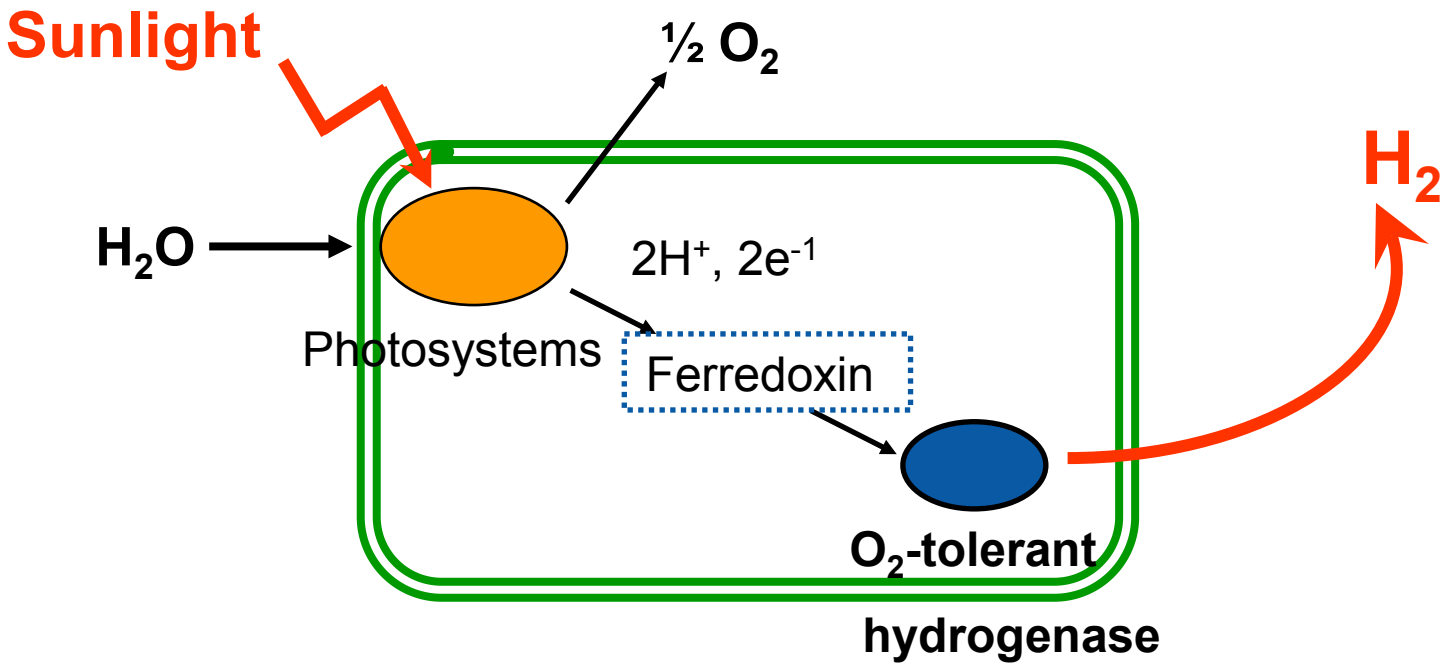
- Barriers addressed
  - Production Barrier Z: Continuity of H_2 production

Partners

- J. Craig Venter Institute
- National Renewable Energy Laboratory

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)

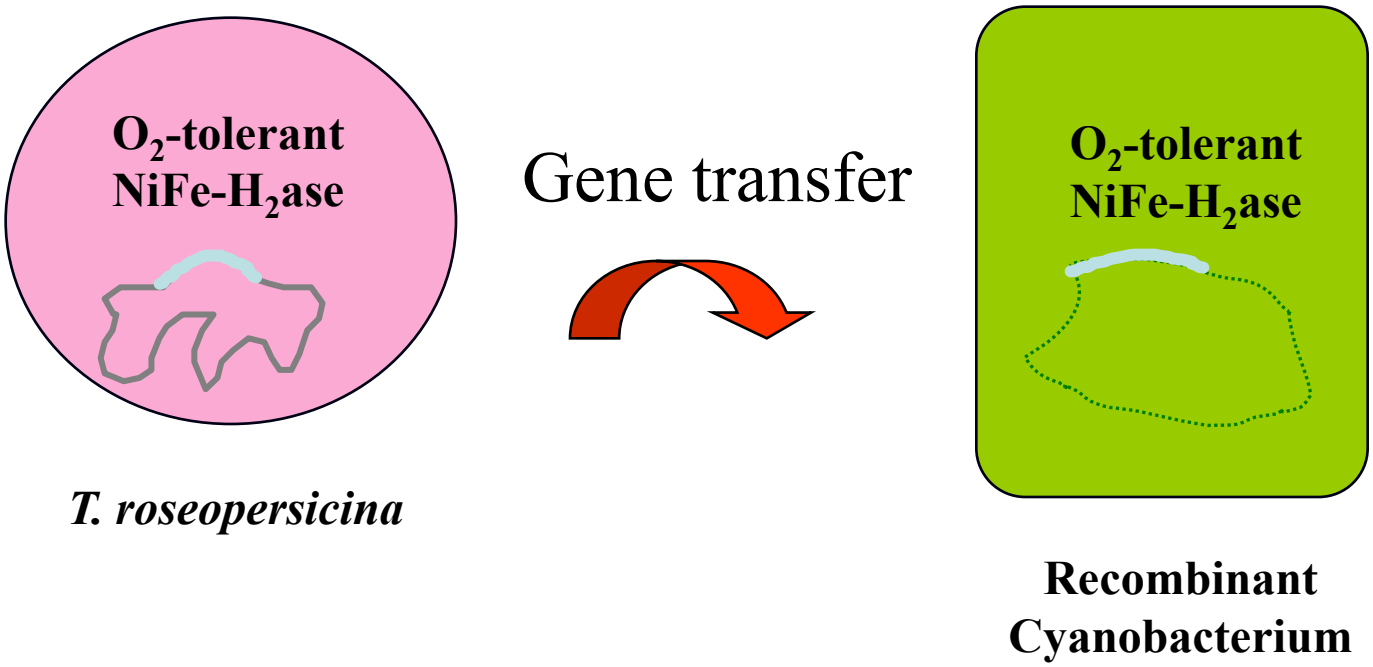
Month/Year	Milestone	% Comp
Sept-09	Purify hydrogenases	JCVI, 100% NREL, 100%
Apr-10	Determine electron mediator requirement	JCVI, 50% NREL, 50%
Sept-09	Verify hydrogenase functionality in oxygen	JCVI, 100% NREL, 100%
Apr-10	Construct cyanobacterial hybrid to express an active *Thiocapsa* hydrogenase	JCVI, 90% 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%
Apr-10	Construct a cyanobacterial hybrid to express an active environmental hydrogenase	JCVI, 50%

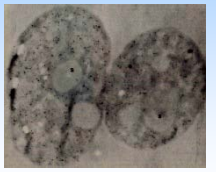
JCVI-Technical Approach

Task 1.1. Transferring a known O_2-tolerant NiFe-hydrogenase from *T. roseopersicina* into cyanobacterium *Synechococcus sp* PCC7942

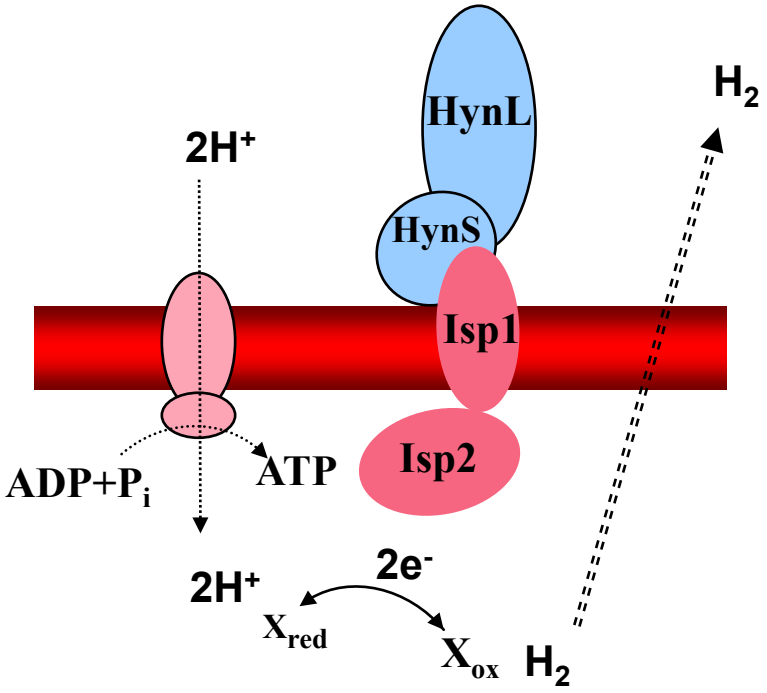


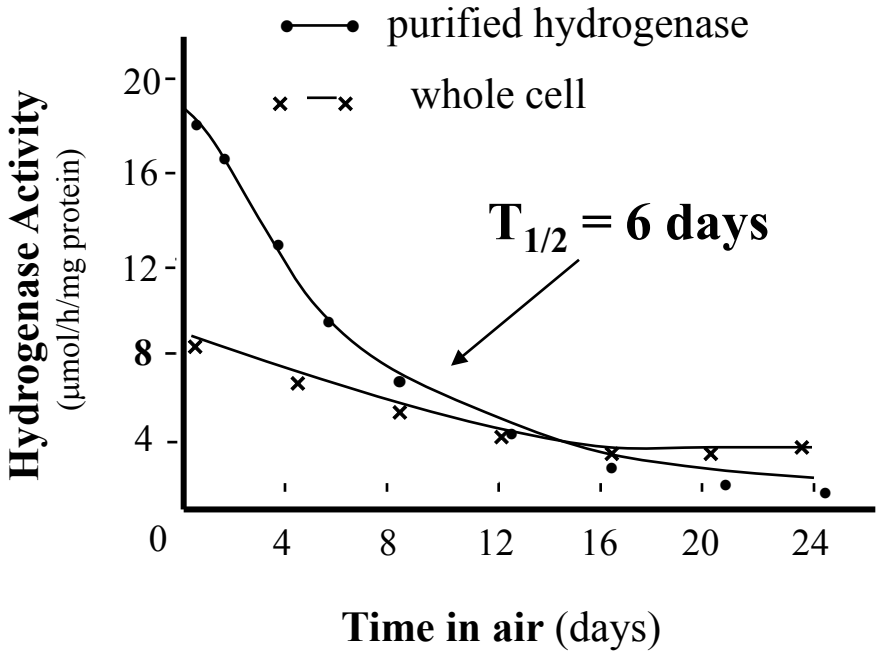
- JCVI approach is complementary to that of NREL in harnessing two of Nature's O_2-tolerant hydrogenases and their transfer into two model cyanobacteria

Phototrophic purple sulfur bacteria *Thiocapsa roseopersicina* carries an O_2-tolerant hydrogenase (HynSL)



- Phototrophic purple sulfur bacterium *Thiocapsa roseopersicina* carries an O_2-tolerant and thermal-stable hydrogenase (HynSL).
- The *Thiocapsa* hydrogenase HynSL displays a half-life of 6 days in air.
- Structural and accessory genes encoding the *Thiocapsa* hydrogenase are identified.





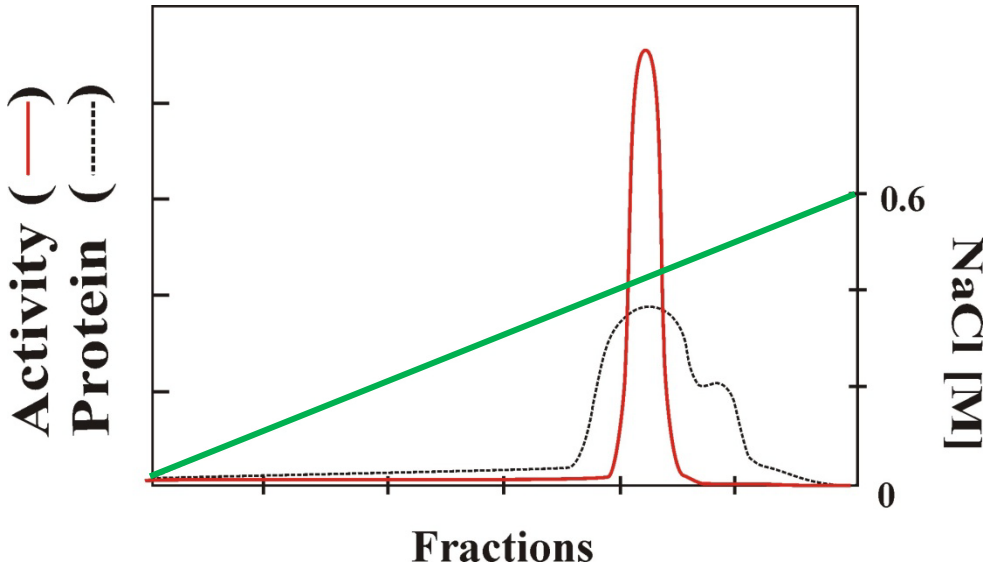
- Structural subunit: HynS and HynL
- Electron transfer subunit: Isp1 and Isp2

Biochimica et Biophysica Acta 523:335-343 (1978)

JCVI - Technical Accomplishments

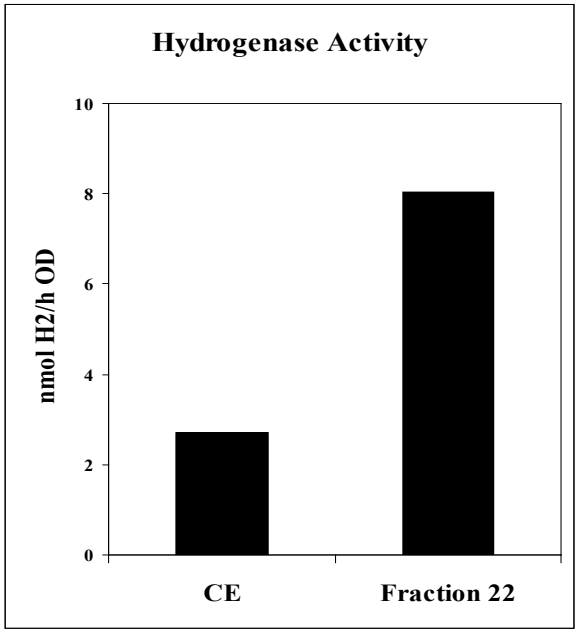
Purified *Thiocapsa roseopersicina* O_2-tolerant hydrogenase

Chromatography of *Thiocapsa* crude extract and H_2 evolution activity assay



- Red = H_2 evolution activity
- Black = Protein concentration
- Green=NaCl gradient 0 - 0.6 M

(The hydrogenase was eluted at NaCl ≈ 0.4 M)

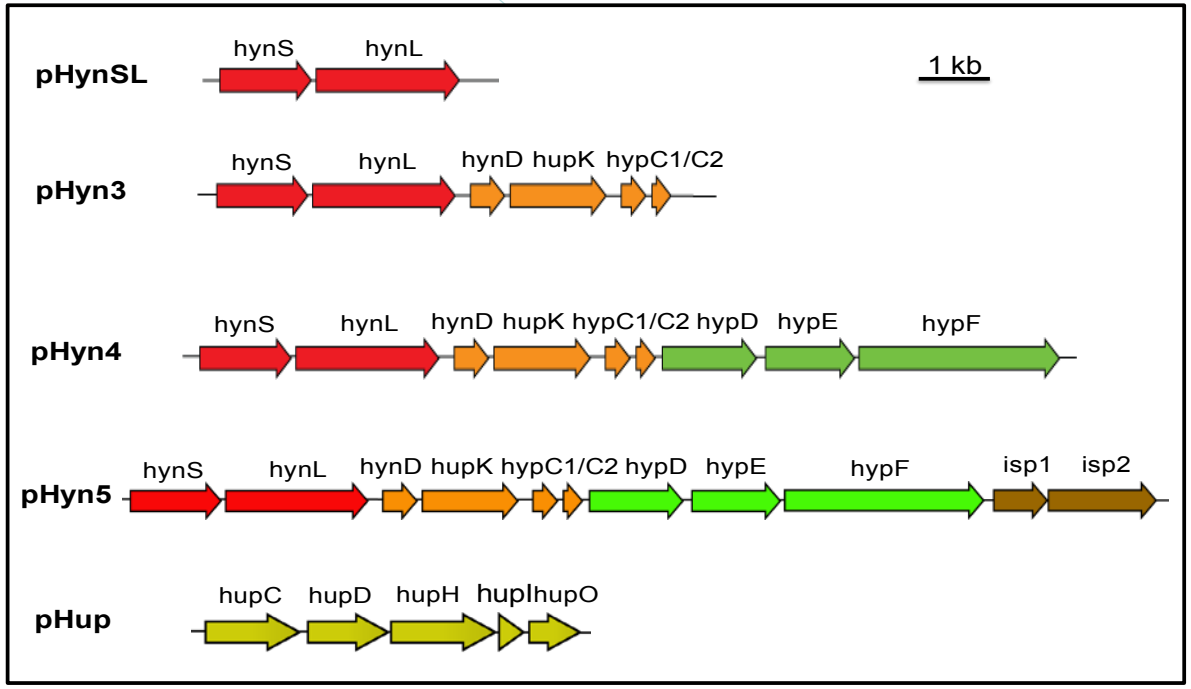


- CE: Crude extract
- Fraction 22 = Purified hydrogenase

Reached Milestone "Purify *Thiocapsa* O_2-tolerant hydrogenases" **(09/09)**

JCVI - Technical Accomplishments

Transferred all genes of the *Thiocapsa* O_2-tolerant hydrogenase (Hyn) into *Synechococcus* PCC7942 Hoxmut



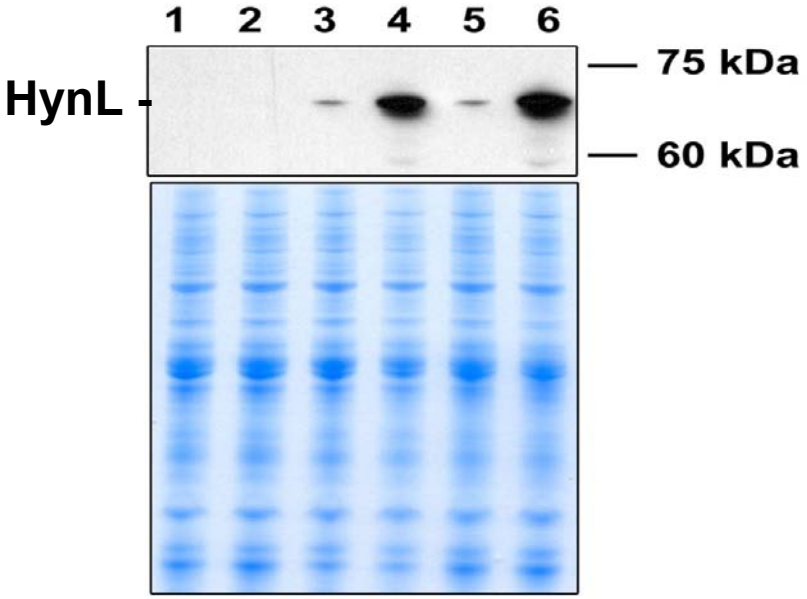
- Structural genes: *hynS/hynL* (red); Electron transfer subunit genes: *isp1* and *isp2* (brown)
- Accessory genes: *hynD/hupK/hypC1/C2 (orange), hypD/E/F* (green) and *hupC/D/H/I/R* (yellow)

- Through homologous DNA recombination, genes in different combinations were integrated into the cyanobacterial genome, as confirmed by PCR and Southern.
- The *Thiocapsa* hydrogenase is expressed under the control of an IPTG-inducible promoter in the mutant strain Hoxmut, in which the native hydrogenase Hox was knocked out.

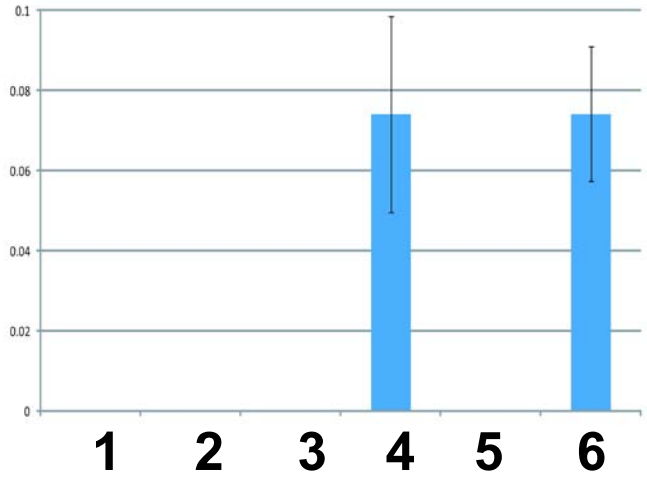
JCVI - Technical Accomplishments

Heterologously expressed an active O2-tolerant Thiocapsa hydrogenase in the recombinant cyanobacterium S. e. PCC7942

A. Western Blotting



B. H2-evolution activity assay



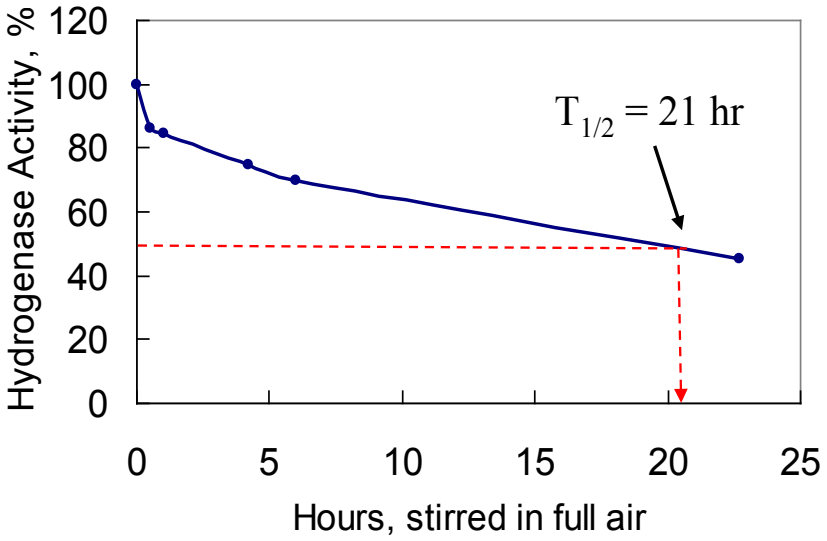
1. Hoxmut – IPTG; **2.** Hoxmut +IPTG; **3.** Recombinant strain (1) –IPTG; **4.** Recombinant strain (1) +IPTG; **5.** Recombinant strain (2) –IPTG; **6.** Recombinant strain (2) +IPTG

- IPTG-inducible expression (A) of O_2-tolerant hydrogenase Hyn was detected in the recombinant *Synechococcus* using specific antibodies.
- IPTG-inducible hydrogenase activity (B) was detected by in vitro H2-evolution assay.

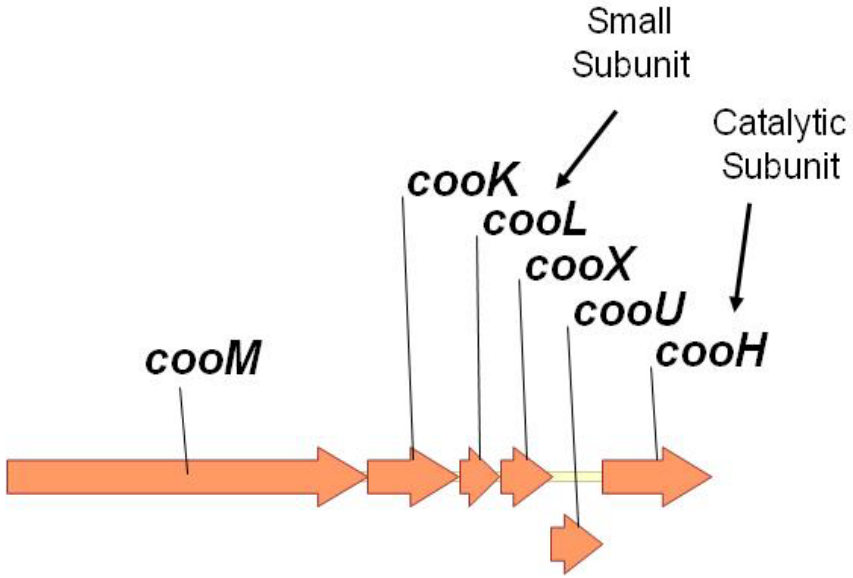
Approach (NREL)

Task 1.2. Transfer an O_2-tolerant NiFe-hydrogenase from the bacterium *Rubrivivax gelatinosus* CBS (hence "CBS") (isolated by NREL) into the cyanobacterium *Synechocystis* sp. PCC6803

CBS Hydrogenase half-life in air: 21 hr



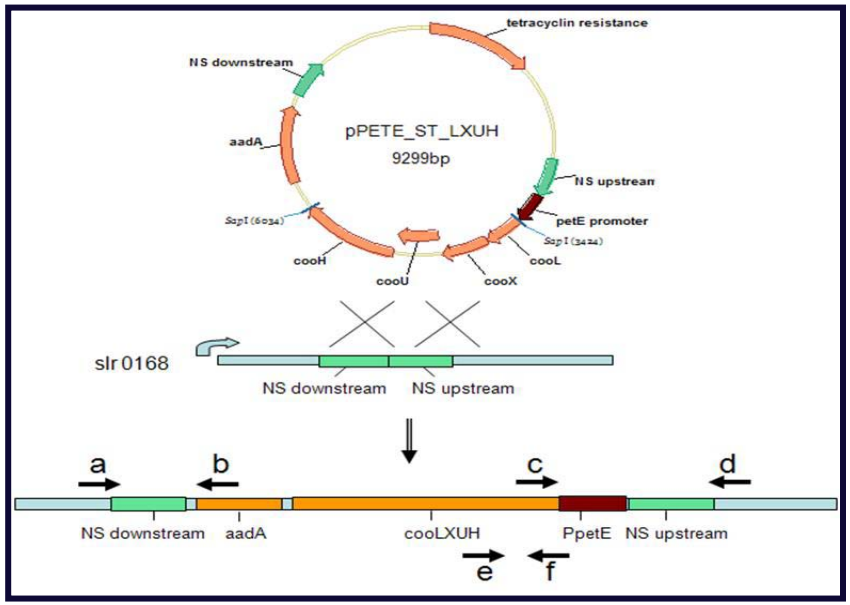
Hydrogenase genes were cloned

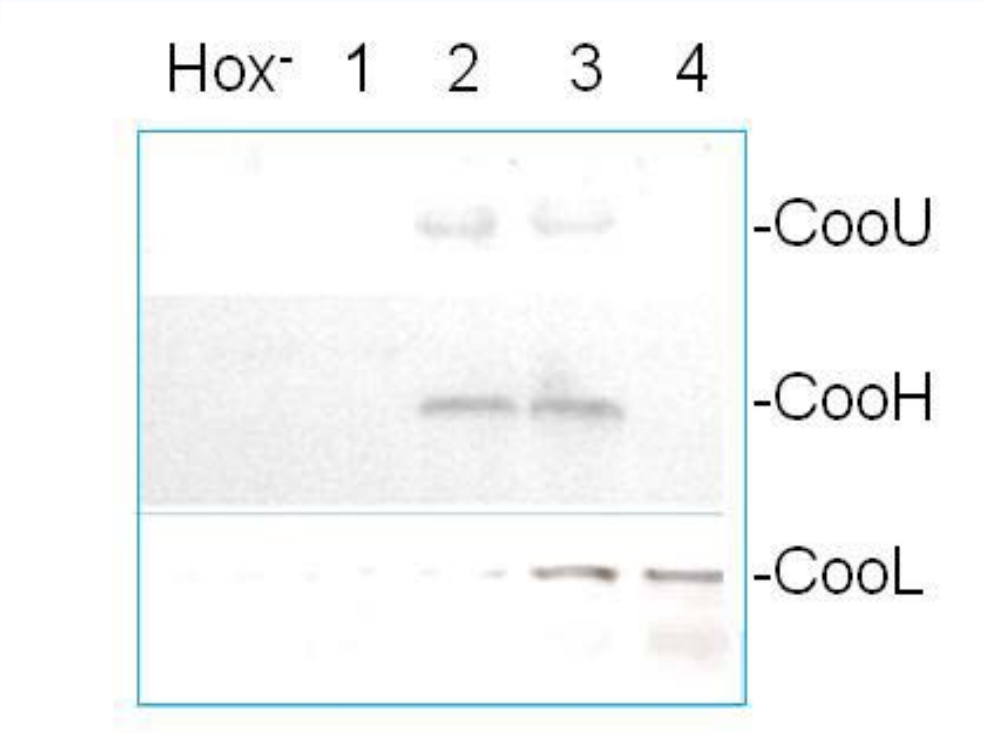


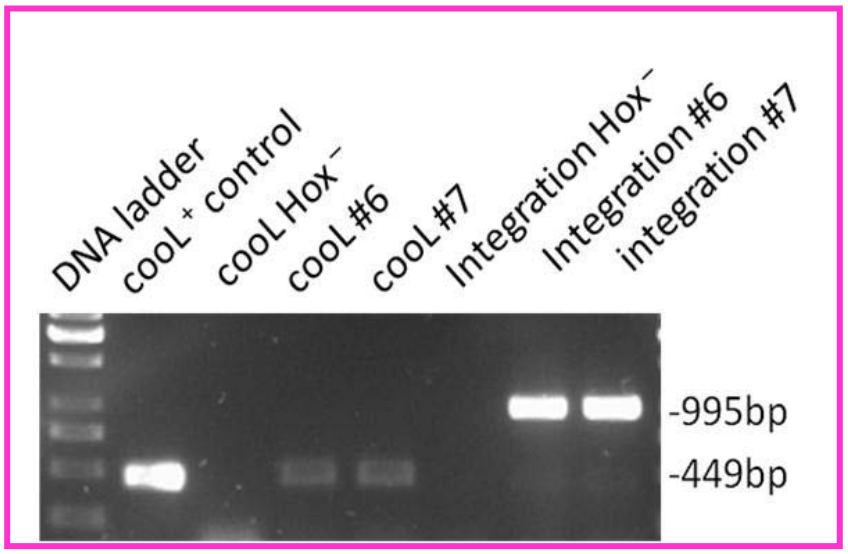
NREL approach is complementary to that of JCVI in harnessing two of Nature's O_2-tolerant hydrogenases and their transfer into two model cyanobacteria

NREL – Technical Accomplishments

CBS hydrogenase expressed in *Synechocystis*



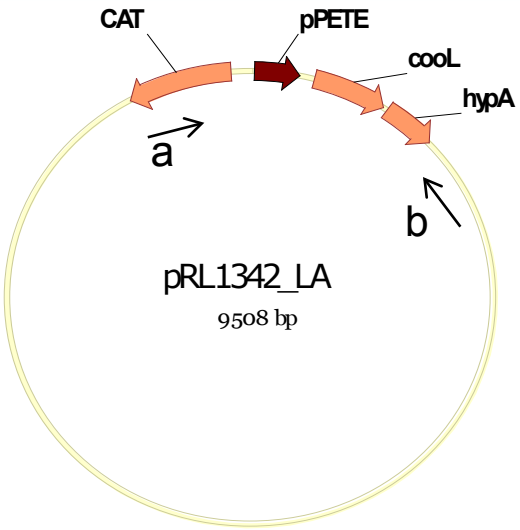


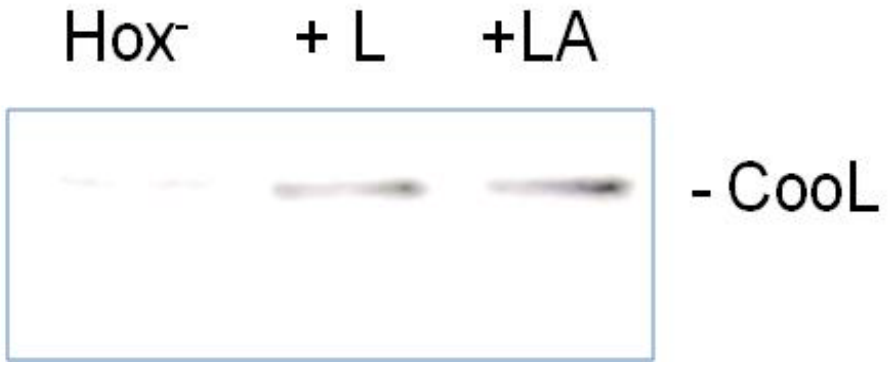


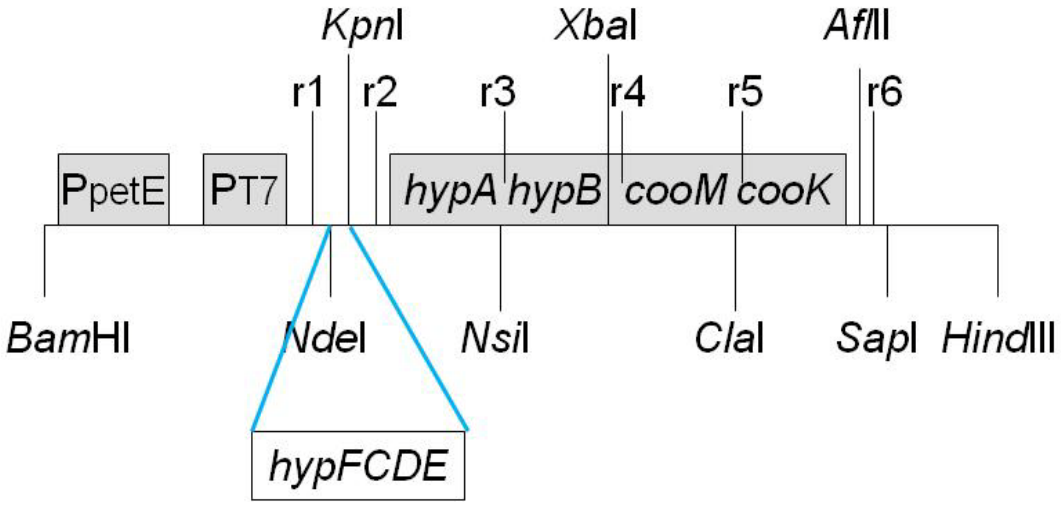
- Obtained *Synechocystis* transformants in the Hox$^-$ zero-H_2 background host.
- Three CBS hydrogenase subunits (CooLUH) were expressed with gene integration in *Synechocystis* via homologous recombination.

NREL – Technical Accomplishments

- **Hydrogenase Maturation Gene Expressed in *Synechocystis***





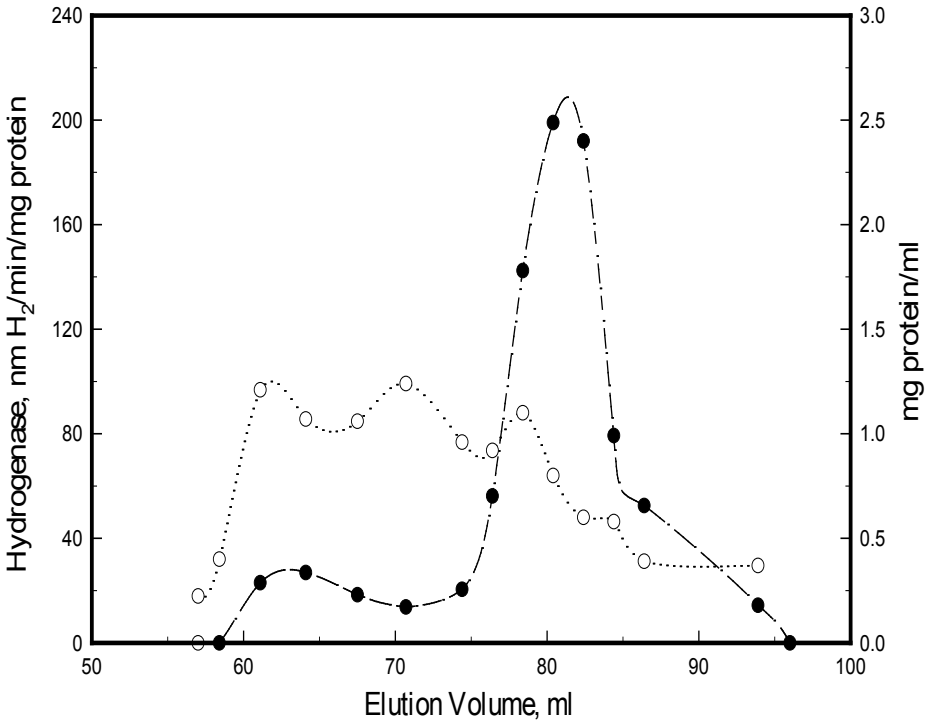


- Constructed a plasmid-based expression system with CooL and likely HypA (maturation) proteins expressed.
- Hydrogenase maturation genes *hypABCDEF* were cloned for expression.

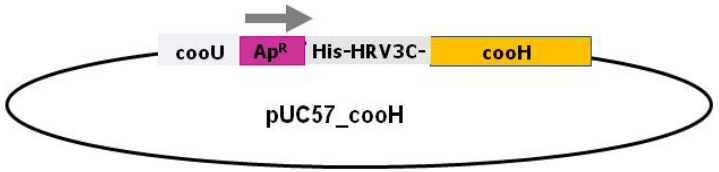
NREL – Technical Accomplishments

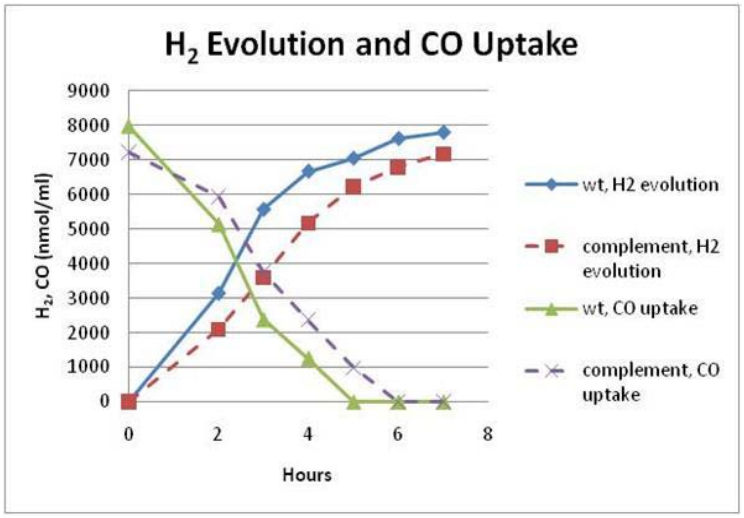
Purified O_2-tolerant CBS Hydrogenase

Size Exclusion Chromatography (Sephacryl S-200)



Develop an affinity purification system





27 fold purification, 5.7% yield

Purified hydrogenase retained 60% activity in 13% O2.

Completed Milestone "*Purify CBS O_2-tolerant hydrogenase* (9/09)

JCVI - Technical Approach

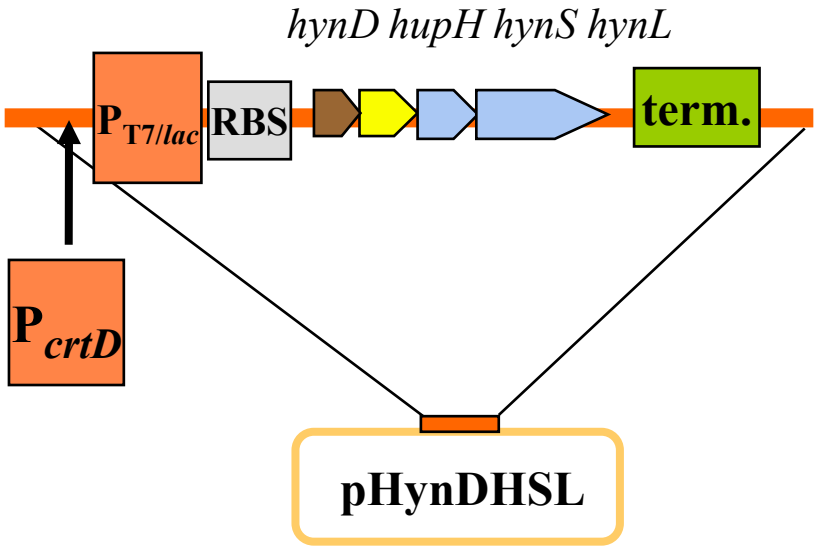
Task 2. Identifying novel O_2-tolerant hydrogenases through metagenomic analysis of marine microbes in the global ocean and transferring the hydrogenases into cyanobacteria

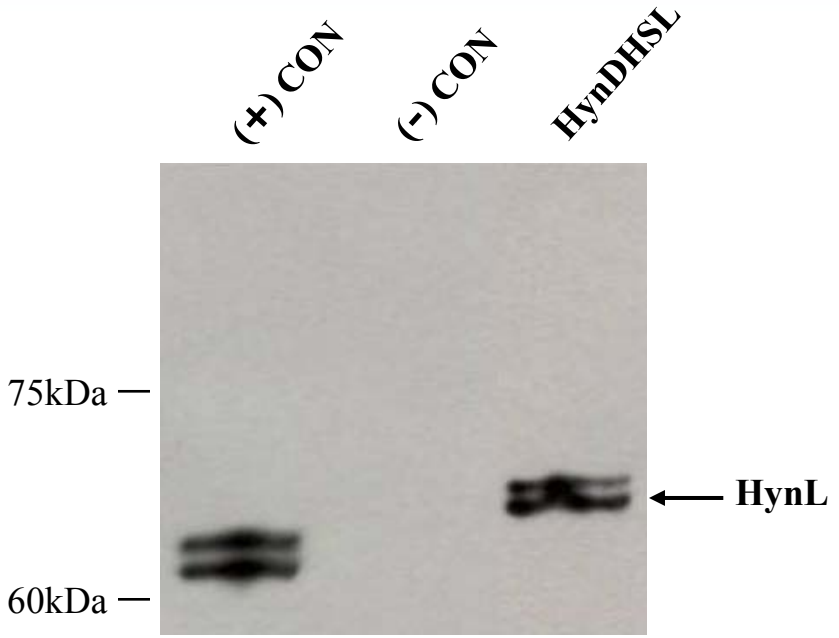
Sorcerer II Expedition: a Global Ocean Sampling Project accomplished by JCVI

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

JCVI - Technical Accomplishments

Cloned and expressed the genes of a novel environmental NiFe-hydrogenase with 60% similarity to *Thiocapsa* O_2-tolerant hydrogenase





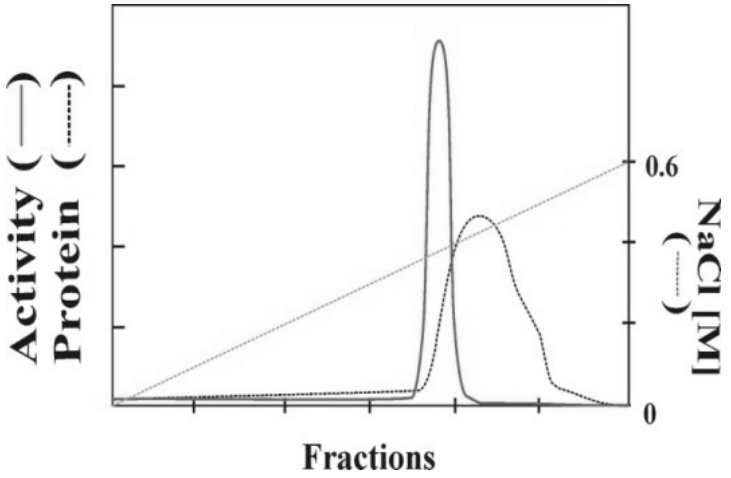
- *hynS*/hyn*L*: hydrogenase structural genes
- *hynD/hupH*: hydrogenase accessory genes
- P_{crtD}: a promoter from *T. roseopersicina*

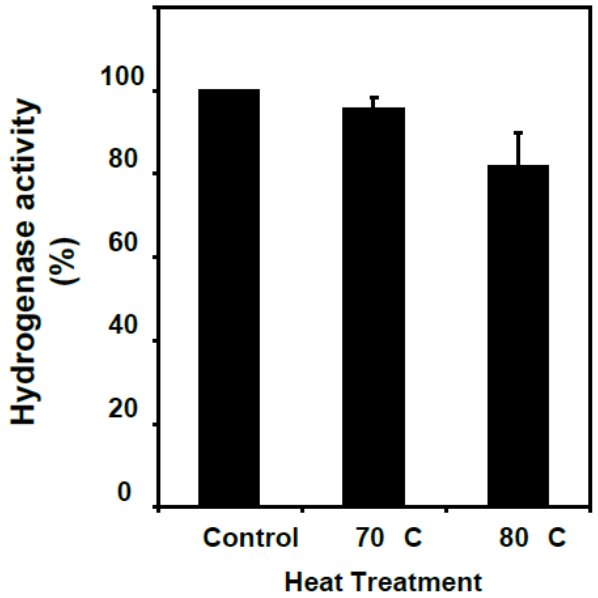
(+) CON: *T. roseopersicina* wild-type strain
(-) CON: *T. roseopersicina* knockout mutant
HynDHSL: *T. roseopersicina* with pHynDHSL

- Structural and accessory genes of a novel hydrogenase were cloned from environmental DNA.
- The construct pHynDHSL carrying *hynD*/*hupH*/*hynS*/*hynL* was transferred into *T. roseopersicina*
- Expression of transferred hydrogenase genes is controlled by *T. roseopersicina* promoter P_{crtD}.
- Western blotting detected expression of the novel hydrogenase HynL in the foreign host of *T. roseopersicina.*

JCVI - Technical Accomplishments

Analyzed the Novel Hydrogenase that was Heterologously Expressed in the *T. roseopersicina* Host

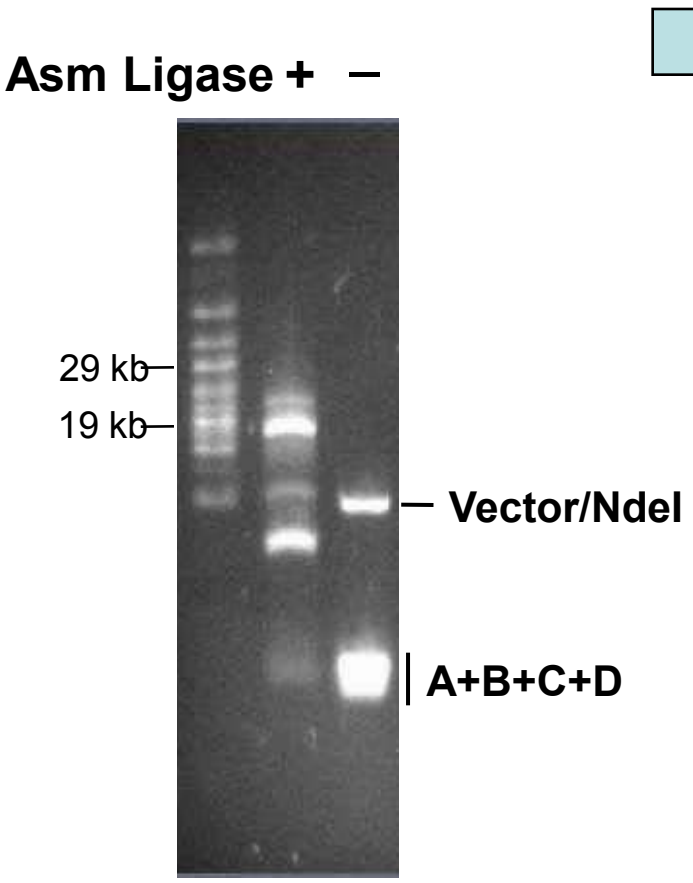


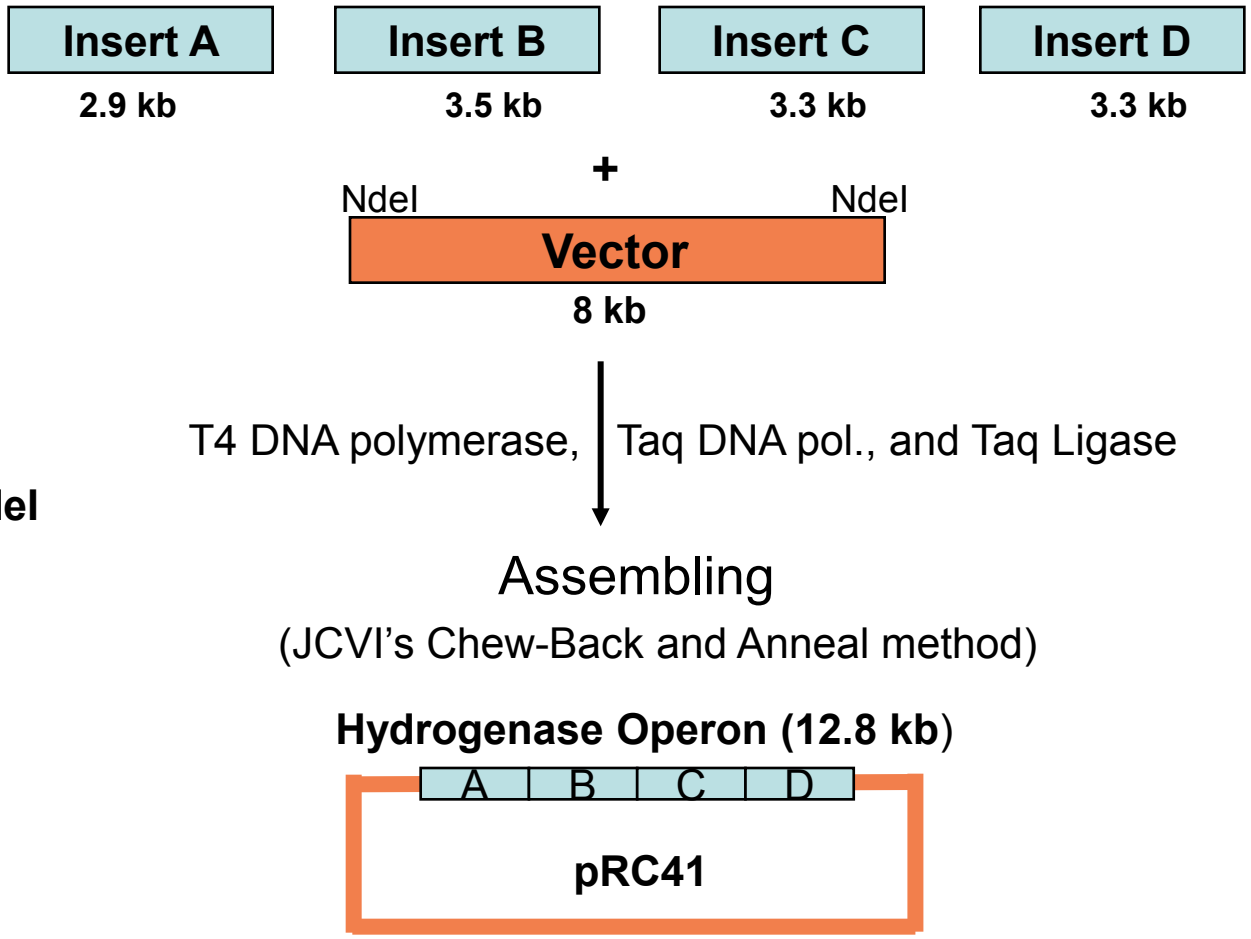


- After purification, the activity of the novel environmental hydrogenase was enhanced 14 times
- The novel hydrogenase showed extraordinary thermo-stability.
- The purified hydrogenase retained ~30% activity in 1% O2.

JCVI - Technical Accomplishments

Assembled and cloned the entire gene operon of the novel environmental hydrogenase for transferring into cyanobacterium



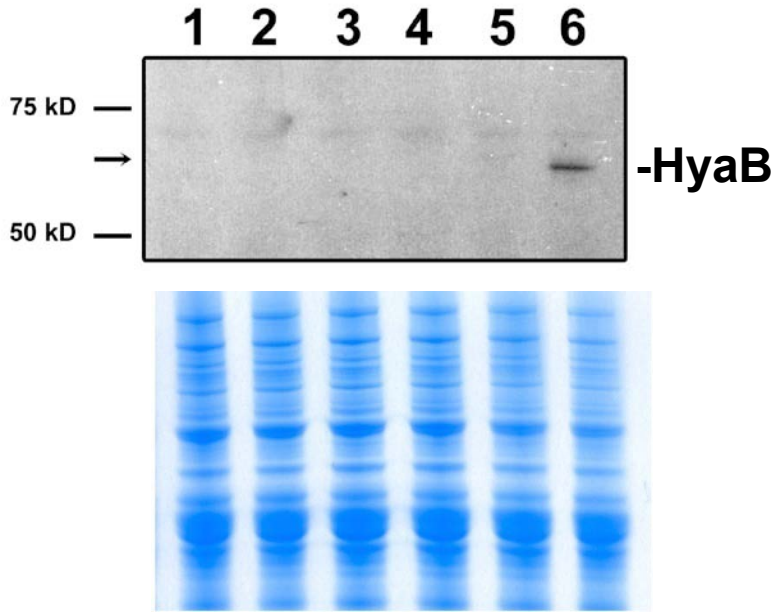


Accuracy of pRC41 was confirmed by RE digestion, PCR, and DNA sequencing. The genes of novel environmental hydrogenase were transferred into *E. coli* and cyanobacterium PCC7942.

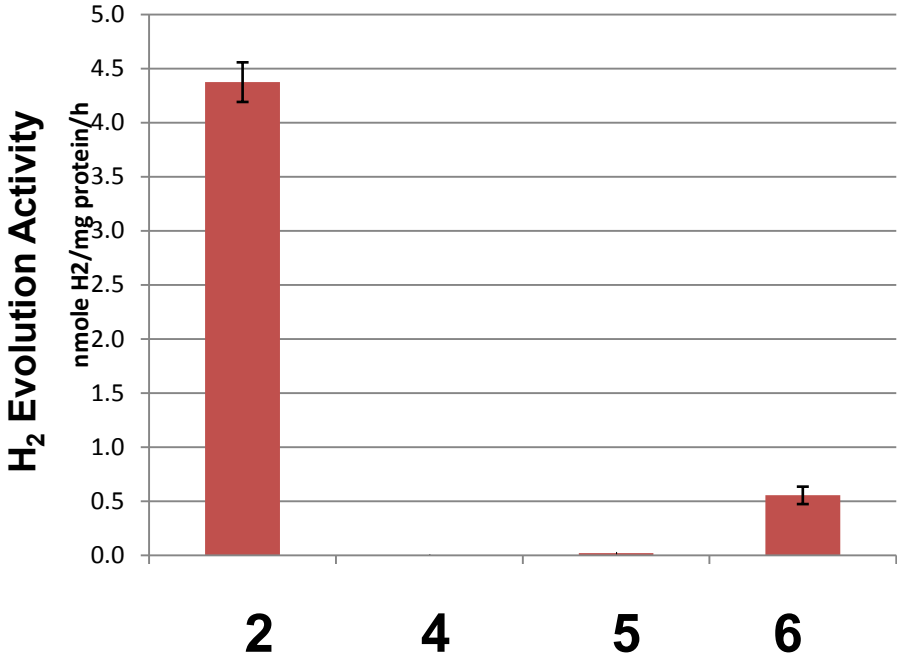
JCVI - Technical Accomplishments

Expressed an active environmental hydrogenase in the host of the cyanobacterium S.e. PCC7942

A. Western Blotting



B. H2-evolution activity assay



1. WT PCC7942 -IPTG; **2.** WT PCC7942 +IPTG; **3.** Hoxmut –IPTG; **4.** Hoxmut +IPTG; **5.** Recombinant cyanobacterial strain –IPTG; **6.** Recombinant cyanobacterial strain +IPTG

- IPTG-inducible expression (A) of novel environmental NiFe-hydrogenase HyaAB was detected in the recombinant *Synechococcus* PCC7942 using specific antibodies.
- IPTG-inducible hydrogenase activity (B) was detected by in vitro H2-evolution assay.

Collaborations

- University of Szeged, Hungary
  - Expressing novel environmental hydrogenase in *Thiocapsa*
- Vanderbilt University
  - Expressing O_2-tolerant hydrogenases in cyanobacteria
- Qingdao Institute of Bioenergy and Bioprocess Technology
  - Sequencing the CBS genome

Proposed Future Work

JCVI

– Re-engineer plasmid constructs and demonstrate increased expression of hydrogenase *in vitro* (FY10 and 11).

– Verify hydrogenase activity in cyanobacteria *in vivo* and assess ability to make hydrogen from water (FY10 and 11).

– Test electron mediator requirement of hydrogenase expressed in cyanobacteria with increased hydrogenase expression (FY10 and 11).

NREL

– Express additional CBS hydrogenase maturation genes and measure hydrogenase activity in *Synechocystis* host (FY10 and 11).

– Begin purification of the affinity-tagged CBS hydrogenase to test its functionality in O_2 (FY10 and 11).

Summary

- **JCVI**

1. The O_2-tolerant hydrogenase from *Thiocapsa* was purified through FPLC. Its linkage with cyanobacterial ferrodoxin has been confirmed *in vitro*.
2. The genes of the *Thiocapsa* O_2-tolerant hydrogenase were transferred into *S.* PCC7942 and activity from the heterologously-expressed hydrogenase was detected.
3. A novel NiFe-hydrogenase was cloned from the Sargasso Sea environmental DNA, expressed *in T. roseopersicina*, and showed activity in the presence of low levels of 1% oxygen.
4. The genes of this novel hydrogenase were transferred into *E. coli* and *Synechococcus*, and activity from the heterologously-expressed hydrogenase was detected.

- **NREL**

1. Developed two different expression systems and expressed at least three CBS hydrogenase subunits and one maturation subunit in *Synechocystis.*
2. CBS native hydrogenase was purified. Developed an affinity system that enables faster purification of CBS hydrogenase for characterization.