Air Force Office of Scientific Research

The Basic Research Manager of the Air Force

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AFOSR MURI Update June 2005-Jan 2006 :

Renewable Bio-solar Hydrogen Production from Robust Oxygenic Phototrophs

BioSolarH_2 → Team

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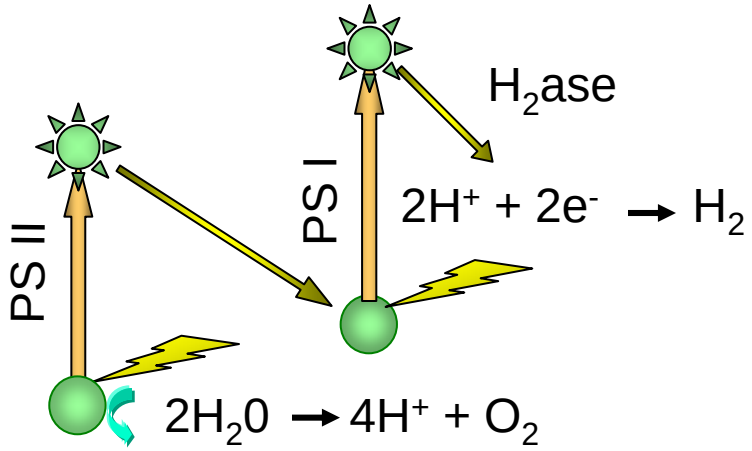
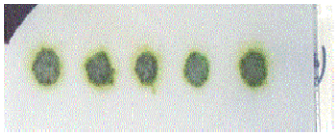
-BioSolarH_2→

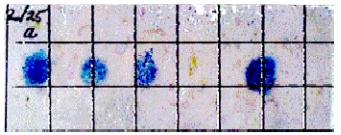
Goal 1: High Throughput Screening for BioSolar H_2 Production



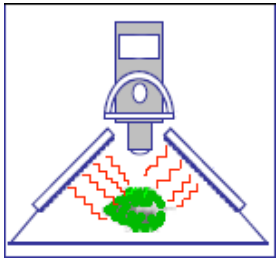
Colonies on agar plates



Chemochromic H_2 sensor



Kinetic Fluorescence CCD Camera



Goal: screen libraries of phototrophs in search of most active H_2 producers using multiple sensors for H_2, photosynthetic capacity and O_2 consumption

Payoff to Air Force

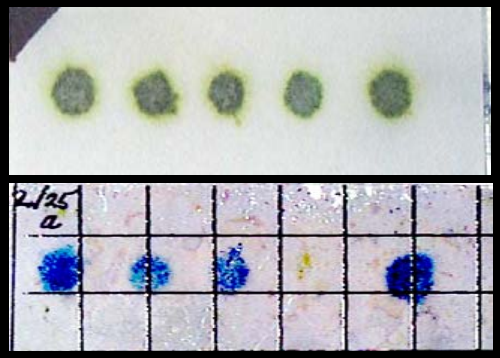
- development of robust screening protocol to identify unique H_2 producing organisms
- development of clean renewable hydrogen fuel

Goal 2: Design/Fabricate Powerful Instrumentation

Current Tools for:

- high throughput screening of gaseous H_2 production
- Light induced electron transfer rates in/out of PSII: $H_2O \rightarrow O_2$, $Q_A^- \rightarrow Q_A$
- Intracellular redox status PSII: PQ/PQH_2
- Nanomolar sensitivity for dissolved O_2 and H_2 concentrations

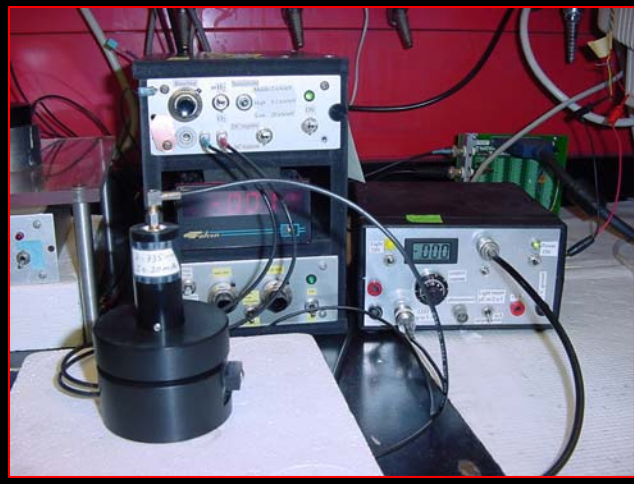
Colonies on agar

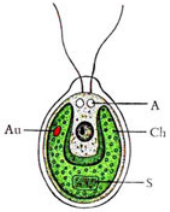


NREL H_2 gas sensor

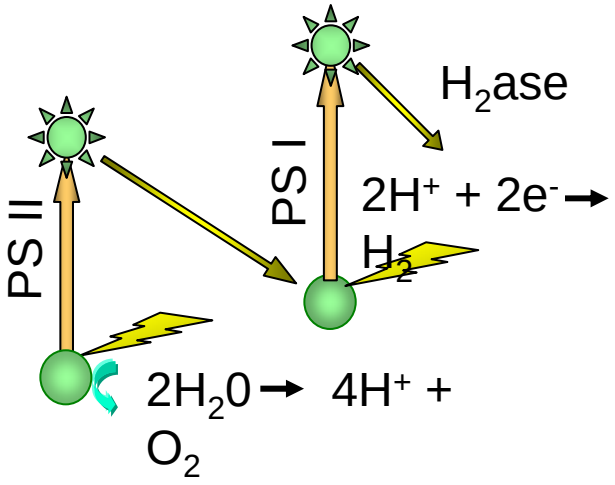
Fast repetition rate fluorimeter
PSII Quantum Efficiency

Dissolved H_2 & O_2
LED + Clark cells





Goal 3: Elucidate Pathways for e^- & H^+ fluxes in Microalgae: Light-Induced H_2 Production in *Chlamydomonas reinhardtii*



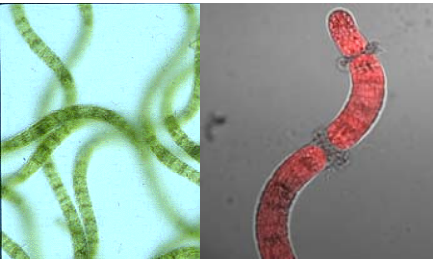
Mechanistic Pathway in WT strain cc124

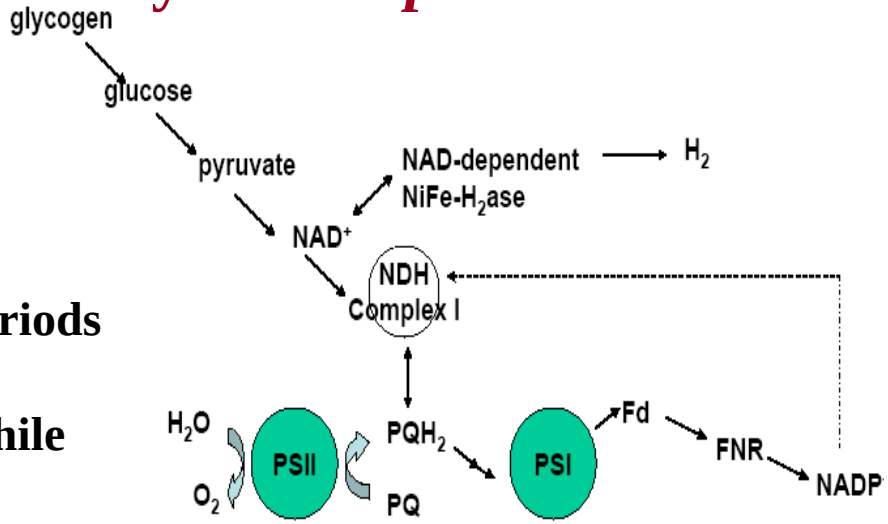
- **Two pools of photo-electron acceptors in PSI identified as precursors to photo-H_2**
- **Established conditions for absence of competing pathways to photo-H_2**

Genetically Engineered Strains

- **Expression of a single set of H_2ase assembly genes has been demonstrated to be sufficient to assemble a diverse set of [FeFe]-hydrogenase structural enzymes from foreign *HydA* genes taken from clostridial bacterial strains.**

Goal 3: Elucidate Pathways for e^- & H^+ Fluxes in Cyanobacteria: Dark Fermentative H_2 production by *Arthrospira maxima*





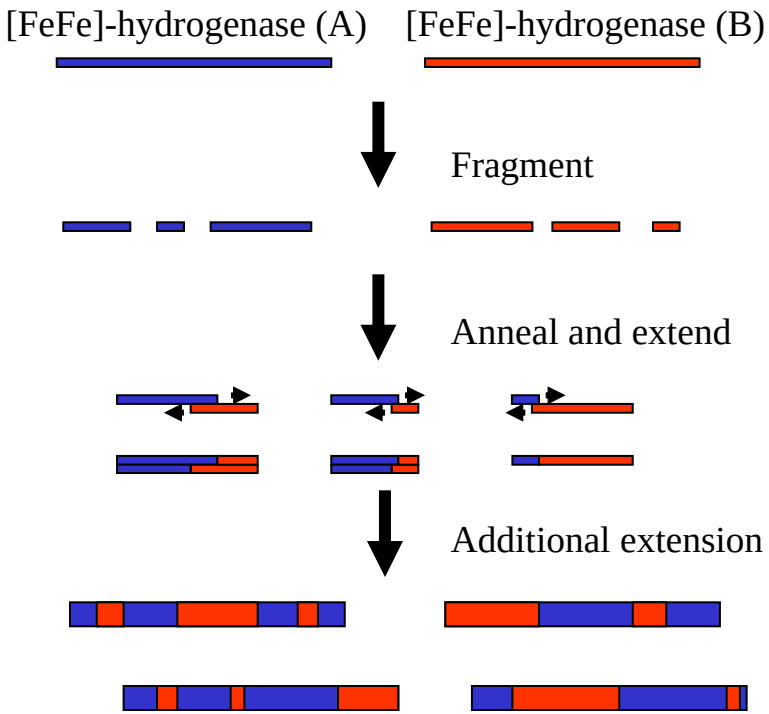
- **Robust photoautotrophic growth over long periods**
- **High carbonate-requiring hypersaline alkalophile**
- **Optimal temporal separation of H_2 and O_2**
- **High rates of fermentative H_2 production optimized by environment conditions (T, pH) and micro-nutrient optimization (Ni, Fe, trace metals): 5.5 ml H_2 (liter culture)$^{-1}$ hour^{-1}**

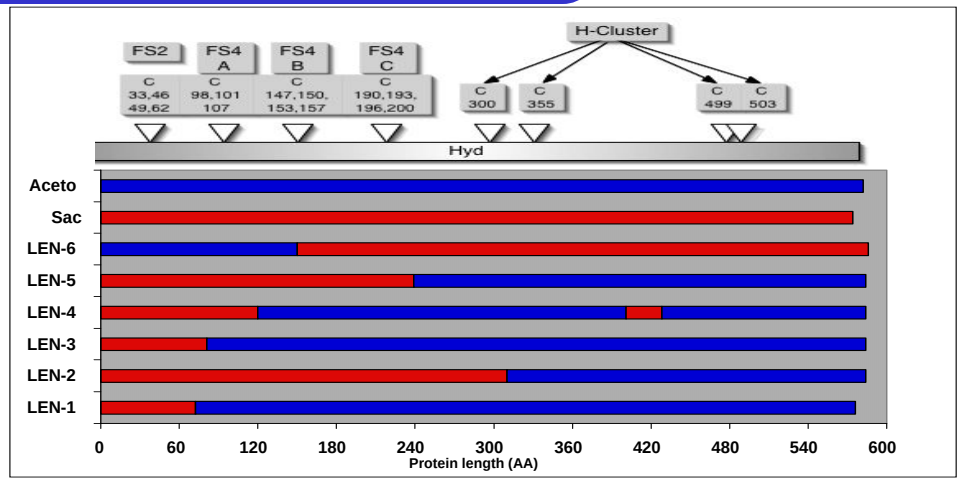
Two stage indirect pathway to generate H_2 via a O_2-tolerant NiFe-H_2ase:

1) **classical photosynthetic generation of glycogen,**
2) **fermentation of glycogen**

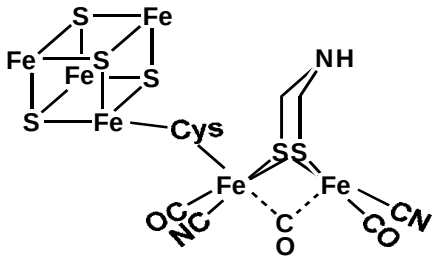
Goal 4: Gene shuffling to rapidly increase diversity of [FeFe]-hydrogenases

Goal: Use recombinant DNA technology to rapidly generate large libraries of novel [FeFe]-hydrogenases. Select robust enzymes for application in BioSolar H_2-production applications





Examples of shuffled products with activity



FeFe-hydrogenase catalytic H-cluster

Payoff to Air Force

- Rapid generation of millions of novel [FeFe]-hydrogenases in less than a week
- Efficiently generates enzyme libraries to be screened for more robust properties
  - Established biotechnology for improving enzyme function