Metabolic Engineering of Hydrogen Production in Filamentous Cyanobacteria

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Objective:

Create and test the feasibility of new hydrogen production system based on photosynthetic filamentous cyanobacteria.

System Design:

An indirect photolysis of water (see left panel) will be created. In this scheme, oxygenic photosynthesis and hydrogen production occurs in separated compartments. Water molecules are split via photosynthesis in one compartment with the concomitant evolution of molecular oxygen. Protons and electrons extracted from water molecules are exported to a second compartment where hydrogen evolution occurs.

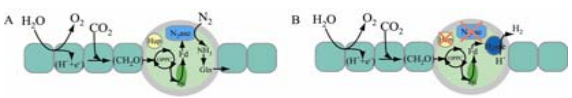
Potential Advantages over Existing Systems:

-Direct conversion of solar energy into chemical energy
-Renewable, carbon neutral
-Potentially higher efficiency than existing methods

Engineering Challenge:

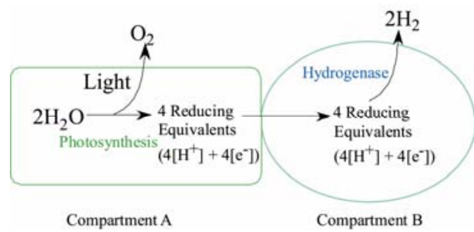
Oxygenic photosynthesis produces molecular oxygen as a byproduct, while hydrogen production via hydrogenase is an anaerobic process. A functional system must therefore be configured as such that the oxygen produced during photosynthesis does not interfere with hydrogen production.

Experimental Approach



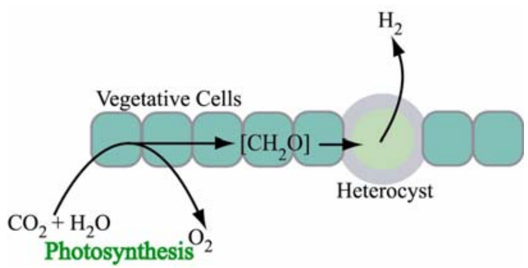
Simplified version of biochemical pathways in (A) normal vegetative cells and heterocysts and in (B) modified heterocysts for hydrogen production. OPPC, oxidative pentose phosphate cycle; Hup, uptake hydrogenase; N_2ase, nitrogenase; H_2ase, hydrogenase; Fd, ferredoxin.

Indirect (2-Stage) Photolysis of H_2O



Goal: To create a indirect/2-stage photolysis system of H_2O in filamentous cyanobacteria. In this system, photosynthesis and hydrogen production occurs in separate compartments (cell types).

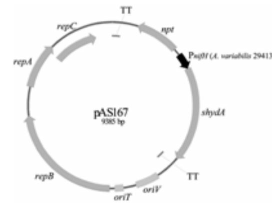
Filamentous Cyanobacterial System



Filamentous cyanobacteria possess an unusual ability to fix dinitrogen while performing oxygenic photosynthesis simultaneously. Under nitrogen limited condition, specialized cells (heterocysts) are developed to fix dinitrogen. Nitrogenase, which reduces nitrogen to NH_3, like hydrogenase requires low $[O_2]$ to function. This provides a ideal solution to the problem of incompatibility between oxygenic photosynthesis and hydrogenase.

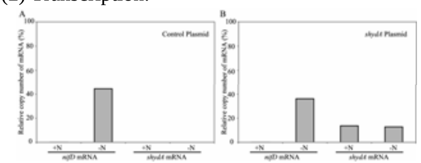
Progress

(1) Expression vector:



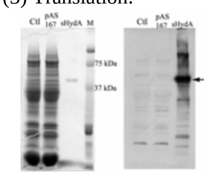
Genetic map of the shydA expression plasmid, pAS167. *repBAC*, replication proteins for RSF1010; TT, transcription terminator; *npt*, neomycin acetyltransferase gene; P*nifH*, nitrite reductase promoter from *A. varibilis* 29413; *shydA*, synthetic hydrogenase gene; *oriV*, origin of replication (RSF1010); *oriT*, origin of transfer (RSF1010).

(2) Transcription:



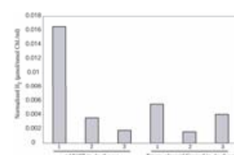
Relative levels of *nifD* and *shydA* transcripts from real-time RT-PCR. (A) *Anabaena* 7120 cells with empty plasmid control. (B) *Anabaena* 7120 cells with the *shydA* expression plasmid, pAS167

(3) Translation:



Western blot analysis of protein extracts from the control with empty plasmid and pAS167 carrying cells. Left panel: SDS-PAGE gel stained with Coomassie R-250. Right panel: Corresponding Western blot probed using polyclonal antibodies against sHydA. Ctl, cell extract from cells with empty plasmid; pAS167, cell extract from cells expressing *shydA*; sHydA, purified sHydA protein; M, molecular weight markers. Arrows indicate the sHydA band. The purified sHydA contains other unknown aggregates that does not bind Coomassie R-250.

(4) Transcription:



Hydrogen production from *Anabaena* 7120 ex-conjugant carrying the hydrogenase expression plasmid, pAS167 and empty plasmid (Controls).