Metabolic Engineering of Hydrogen Production in Cyanobacterial Heterocysts

Investigators

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Introduction

Hydrogen holds great promise to be the energy carrier for the fuel cell-based economy in coming years. Currently, the production of hydrogen relies mainly on the chemical reforming of gasoline. A major drawback of this process is that carbon dioxide is produced as a waste product. To help curb the emission of greenhouse gases, the ideal hydrogen source should be renewable and carbon-neutral. To address the technological challenge for such a renewable carbon-neutral source of hydrogen, we are investigating the feasibility of using photosynthetic bacteria to produce molecular hydrogen from water using light as the energy source.

Main Goals:

1) Modify the metabolism of existing photosynthetic cyanobacteria to produce molecular hydrogen using light energy;
2) Determine the efficiency of the light-driven hydrogen production;
3) Identify rate-limiting step(s) in the light-driven hydrogen production and devise “fixes” to improve the light conversion efficiency.

Experimental Design:

Photosynthesis is used to capture and transform light energy into chemical forms available for the reduction of protons to molecular hydrogen. The design, however, is far from straight forward since photosynthesis (specifically oxygenic or oxygen-evolving photosynthesis) is incompatible with hydrogen evolution. Hydrogenases, enzymes that catalyse the reduction of protons to molecular hydrogen, are extremely sensitive to oxygen and are quickly inactivated in the presence of oxygen. The design challenge therefore involves finding a way to preserve hydrogenase activity in the presence of oxygenic photosynthesis.

Solution to the above problem can be found in nature where oxygen-sensitive processes exist along side with oxygenic photosynthesis. One of the best examples is found among filamentous cyanobacteria which perform both oxygenic photosynthesis and nitrogen fixation simultaneously. Like hydrogenases, nitrogenases (enzymes responsible for reducing dinitrogen to ammonia) are highly sensitive to oxygen. Filamentous cyanobacteria solve this problem by restricting the distribution of nitrogenase only in specialized cells called heterocysts. Within heterocysts, oxygenic photosynthesis is suppressed. In effect, filamentous cyanobacteria spatially separate photosynthesis and nitrogen fixation to accommodate the oxygen-sensitive nitrogenase.

Our plan is to use this existing arrangement to our advantage to produce molecular hydrogen. Hydrogenase will be expressed in heterocysts.

Results

An expression vector has been constructed to express a Fe-only hydrogenase specifically in heterocysts. This vector was conjugated (bacterial mating) into the cyanobacterium *Anabaena*. Hydrogen production was examined with a batch culture assay of the transconjugants. Preliminary analysis suggests and increase in hydrogen production. We are adding further modifications to enhance the yield of the cyanobacterium. Once the final strain is constructed, the photo-conversion efficiency with respect to the hydrogen production will be measured.