Photobiological Hydrogen Research

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Abstract

The goal of this R&D project is to identify the structural and active site maturation genes of an O2-tolerant flavin hydrogenase, which are critical to optimal expression of the enzyme in E. coli. This information is essential for future genetic engineering and expression optimization. The overall approach used was large-scale screening of our internal bacterial collection, including genetically engineered strains formed during our hydrogen production efforts. The challenge for this project is to identify the maturation genes and construct plasmids expressing the full hydrogenase. The goal is to construct a recombinant hydrogenase which is functional and active in E. coli.

Technical Accomplishments/Progress/Results

Task 1: Overall Clones of the O2-Tolerant [NiFe]Hydrogenase from R. gelatinosus In Duet Vectors.

- The large subunit of the hydrogenase was cloned and transformed into a zero background E. coli.
- The subunit genes, cooL and cooH without strep tag, and the structural genes, cooA and cooB, were cloned and transformed into a Duet expression vector.

Task 2: Double-plasmid transformation of the hydrogenase genes into MCH10PDTS(DEF).

- The cooK subunit of the hydrogenase was cloned and transformed into a zero background E. coli.
- The large subunit of the hydrogenase was cloned and transformed into a zero background E. coli.
- The subunit genes, cooL and cooH without strep tag, and the structural genes, cooA and cooB, were cloned and transformed into a Duet expression vector.

Task 3: Confirmation of the transformation by DNA gel assays.

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Task 4: Detection of the Large Subunit (CooH) in the Harassed CBS Hydrogenase in a Double-Transformed C. coli.

- The large subunit of the hydrogenase was detected in a double-transformed C. coli.

Task 5: Efficiency of the hydrogenase production.

- Efficiency of the hydrogenase production.

Task 6: Detection of the expression of the hydrogenase by western blotting.

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Technical Accomplishments/Progress/Results

Summary

The project achieved the goals as planned, confirming the maturation of the O2-tolerant NiFe hydrogenase from R. gelatinosus C. coli. The project determined the maturation genes and constructed plasmids expressing the full hydrogenase. The project demonstrated the efficiency of the hydrogenase production and confirmed the expression of the hydrogenase by western blotting.

Benefits from this Project

- This project aims at determining the minimum number of auxiliary and structural genes required for the production of a fully functional NiFe hydrogenase from R. gelatinosus C. coli.
- The project will lead to an improved understanding of how solar-driven, water-splitting cyanobacteria can become hydrogen-producing vehicles.
- The molecular manipulations are complete. H2 production via fermentation using E. coli and cyanobacteria will serve as a technology platform for commercialization.

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