Growth and Hydrogen Production of *Rubrivivax gelatinosus CBS*

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**Abstract**

*Rubrivivax gelatinosus CBS* is a purple non-sulfur photosynthetic bacterium that has been found to produce substantial amounts of Hydrogen (H₂) which could be used as a clean burning biofuel. CBS has been known to grow on a variety of carbon sources, but it is unknown as to which carbon sources it has the greatest potential to use, and this understanding could be helpful in engineering future strains of the bacteria. Hydrogen production in CBS occurs when carbon monoixde (CO) is present and is presumed to be under the control of the *rcoM* transcription factor. When CBS wild-type strains were grown in different carbon sources it was found that the bacteria were able to use the organic acid substrates much more successfully than the sugar substrates. This is useful in that organic acids are often waste products and we can use those products as a nutrient to support the growth of CBS. When the *rcoM* gene was deleted from the CBS bacteria, growth with CO in the mutant strain ceased, and H₂ production and protein production were greatly reduced when compared to the wild-type. Understanding how *RcoM* impacts the CO to H₂ pathway will allow us to better engineer the pathway to further increase the production of H₂ in CBS.

**Introduction**

- Hydrogen (H₂) has the capacity to be a future biofuel because it is a clean fuel source and renewable.
- *Rubrivivax gelatinosus* CBS is a purple non-sulfur bacteria that was isolated from the Denver, Colorado area, that has been found to produce substantial amounts of H₂ in presence of carbon monoixde (CO).
- It is unknown which carbon sources CBS has the capability to use.
- CBS has the capability to use CO and convert it into H₂ in the following reaction(s):

  \[ \text{CO} + \text{H}_2\text{O} \rightarrow 2 \text{e}^- + 2 \text{H}^+ + \text{CO}_2 \]
  \[ 2 \text{e}^- + \text{H}_2 \rightarrow \text{H}_2 \]
  \[ \text{CO} + \text{H}_2 \rightarrow \text{CO}_2 + \text{H}_2 \]

- The pathway of converting CO to H₂ is presumed to be under the control of the *RcoM* protein.

**Carbon Sources:**

- Wild-type CBS was used for all growth experiments.
- When CBS was grown photosynthetically under anaerobic conditions, lactate and malate promoted the fastest growth rates. Organic acids were better substrates for growth than sugars (Figure 2A).
- CBS did not show any growth through fermentation.
- Aerobic growths of CBS had immense growth on liria broth and lactate, with malate cultures beginning to grow at 24 hours. Organic Acids were the only carbon sources to show growth; there was no growth in any sugars (Figure 2B).

**Purpose:**

- To determine what carbon sources CBS grows the most substantially on to understand how to engineer CBS strains most successfully.
- To determine if the *RcoM* protein controls the genes related to the CO to H₂ production pathway, in order to produce clean burning biofuels that may be able to use syngas as starting material.

**Materials and Methods**

**Carbon Sources:**

- CBS strains were grown overnight to produce healthy cultures.
- The optical density (OD) (Figure 1A) of overnight cultures were taken, and then spun down and resuspended to OD₆₆₀ of 0.1 in new growth media.
- Once cells were mixed with a particular carbon source (Figure 1B) the OD₆₆₀ was taken at multiple time points and plotted to compare growth.

**Results**

**RcoM:**

- When induced with CO, the ∆RcoM strains stopped growth whereas the wild-type strains were able to use CO as their only carbon source for continued growth. (Figure 2C)
- Hydrogenase assay of CO growths showed that wild-type had high yields of hydrogen compared to mutants (Figure 2D).
- Western blots of proteins associated with the CO pathway showed little to no production of the proteins in the mutant strains. (Figure 2E)

**Discussion/Conclusion**

**Carbon Sources:**

- Photosynthetic growths grew well on malate, lactate, acetate, and also grew on fructose but with a much greater lag time.
- Aerobic growths also grew on malate, lactate, and acetate.
- In both photosynthetic and aerobic experiments growth was much greater in organic acids, but there was no growth seen in any sugars for the aerobic growths.
- CBS does not ferment well on any tested carbon substrate.
- ∆RcoM strains were unable to use CO as a sole carbon source.
- H₂ production is severely reduced in ∆RcoM strains, and presumably RcoM is not present to activate transcription of the hydrogenase genes. This hypothesis is supported by the lack of hydrogenase protein accumulation in the ∆RcoM strains in the presence of CO.

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