Electron Transfer in Microorganisms: Essentiality of the E- and F- [Fe_4S_4] clusters of the *Methanosarcina acetivorans* CODH enzyme

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Abstract

Methanogens are strictly anaerobic archaea that reduce substrates to methane to produce ATP energy.

- The acetyl-CoA decarbonylase synthase/carbon monoxide dehydrogenase (ACDS/CODH) enzyme complex is critical in the conversion of carbon to methane.
- These enzymes are crucial in energy production, and it is proposed that the E- and F- iron-sulfur clusters are critical for enzyme function.
- If these residues were altered or removed to inhibit assembly of the Fe_4S_4, it could potentially impact enzyme catalysis and therefore growth of these organisms unless acetate is supplemented into the growth medium.

Project Idea

The research project aims to answer several questions about pathways within methanogens, such as if removal of the E- and/or F- Iron-sulfur clusters impact the growth and survival of the methanogen, *M. acetivorans*? How severely is the growth of the organism impacted?

If there is growth, what other clusters can compensate for the lack of the E and F clusters function in electron transfer? Are there other potential pathways being utilized to accommodate?

The goal of this project is to better understand the enzyme complex and if methanogens can adapt without this seemingly crucial piece of methanogenesis.

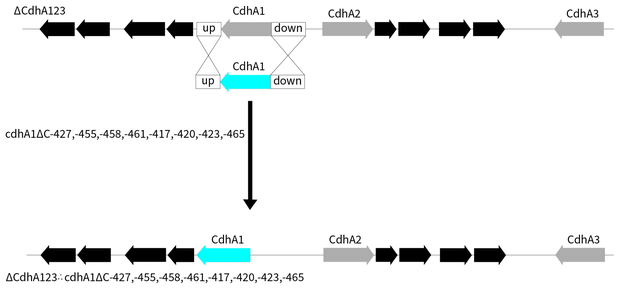

Figure 1: Diagram of the three chromosomal loci on the genome, showing expression of the mutant cdhA1 gene (blue) from the att locus

ACDS/CODH

Acetyl-CoA Decarbonylase Synthase (ACDS) is a multienzyme complex that is involved in the reversible cleavage and synthesis of acetyl-CoA. It is a vital component of methanogenesis and is also involved in the conversion of carbon into methane. Acetyl-CoA is used for either energy or biomass, both of which are essential.

The carbon monoxide dehydrogenase (CODH) enzyme is involved in converting carbon monoxide and water into carbon dioxide and another product, which differs between organisms that utilize this enzyme. Iron-sulfur proteins are found in all life forms. Most frequently, they contain Fe_2S_2, Fe_3S_4, and Fe_4S_4 clusters. They are small inorganic structures involved in the catalytic site of numerous enzymes.

They have significant roles in e- transfer, redox and non-redox catalysis, and DNA/RNA binding. The clusters undergo oxidation-reduction reactions and can influence protein structure. They involve specific protein-binding sites that ligate irons through the cysteine thiolates. The two clusters focused on this research project are E- and F- within CODH, which are proposed to be essential to conduct intermolecular electron transfer to ferredoxin-like proteins.

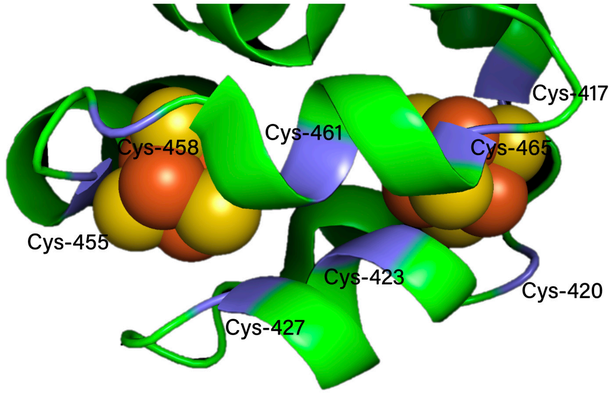

Figure 2: Cysteine residues to be removed using site-directed mutagenesis. Iron-sulfur clusters E- and F- can also be seen in orange/yellow. Cysteine residues are colored in blue.

Strain Construction

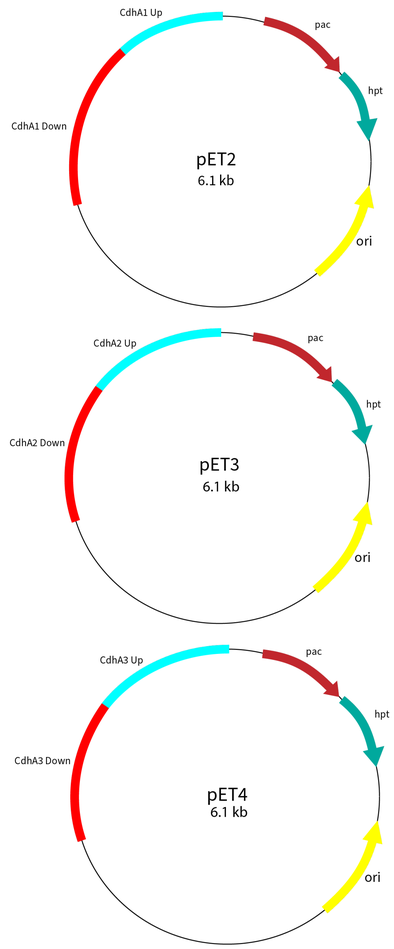

Figure 3: Plasmid maps of the three deletion plasmids in *E. coli* that were created. These will be transformed into *M. acetivorans* followed by the removal of the cysteine residues in both clusters.

Future Work

Once the deletion plasmids are completed, they can be transfected into *M. acetivorans*. Then, we will use site-directed mutagenesis to removed the desired cysteine residues within the cluster binding domain. Once completed, growth will be observed over a month period. Growth curves will be created to determine the effect of the removal of the iron sulfur clusters.

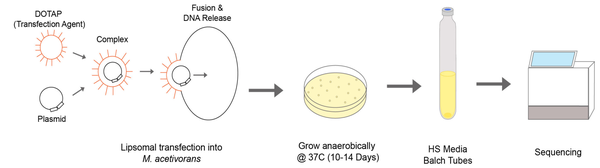

Figure 4: Methods to create and validate strains in *M. acetivorans*. Liposomal transfection is used to transfer plasmids from *E. coli*. Following successful transformation, colonies are grown up in Balch tubes. Strains are validated via PCR and DNA sequencing.

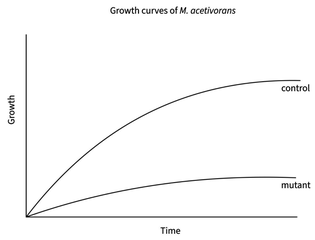

Figure 5: Expected outcomes of the growth curves. Since these clusters are proposed to play a vital role in biomass production via ACDS/CODH, it is hypothesized that mutant strains will have severely impacted growth.

References

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