Cloning and Expression of *MA3299* – An Fe-S Enzyme in the Biosynthesis of Coenzyme M in *Methanosarcina acetivorans*.

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Abstract

Methanogenic Archaea are of special interest because they release methane as a byproduct of their metabolism. The methane is added and then released from a unique cofactor, coenzyme M (CoM). Synthesis of CoM gives methanogens great potential for industrial use; however, the final step remains a mystery. MA3299, a protein found in *Methanosarcina acetivorans*, is believed to catalyze a final step of CoM synthesis, yet it has eluded efficient expression. By working with the native nucleotide sequence as well as removing the unstructured termini, our goal is to finally express, purify, and characterize MA3299.

Research Questions

What can we do to express MA3299 in *Escherichia coli* for characterization?

What is the reaction mediated by MA3299?

Background

Importance of Studying Non-Model Organisms

- Novel metalloproteins
- Biotechnology applications

Methanogens

- Anaerobic Archaea that produce methane from various substrates flowing through the Wolfe Cycle[1]
- A reduced carbon is added to CoM and released[2].
- The final step of CoM biosynthesis remains uncharacterized.

MA3299

- A putative Fe-S protein from *M. acetivorans*, a species found in marine sediments[3].
- Hypothesized to be last step in CoM biosynthesis
- Unstructured regions on the N and C termini and two iron-sulfur cluster binding domains are identified in the AlphaFold (Figure 2).

Current Progress

Homolog Sequence Alignments

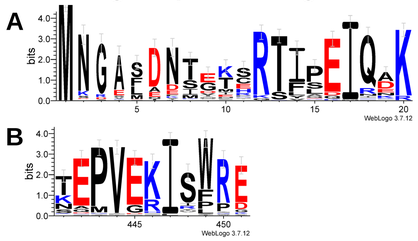

Figure 1. Sequence logos of MA3299 homologs at the MA329 (A) N-terminal (1-20) and (B) C-terminal (428-438) unstructured regions. n = 91.

MA3299 AlphaFold Models

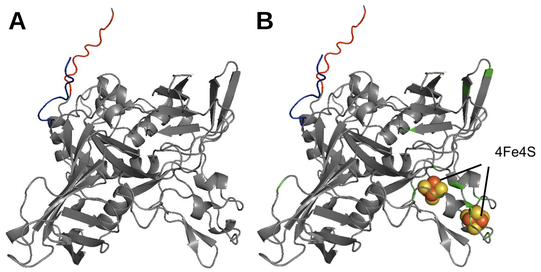

Figure 2. AlphaFold model of MA3299 (A) alone and (B) with Fe-S clusters modeled. N terminus (2-11) in red and C terminus (428-4380) in blue.

Plasmid Design for Expression of 6His*MA3299* in *E. coli*

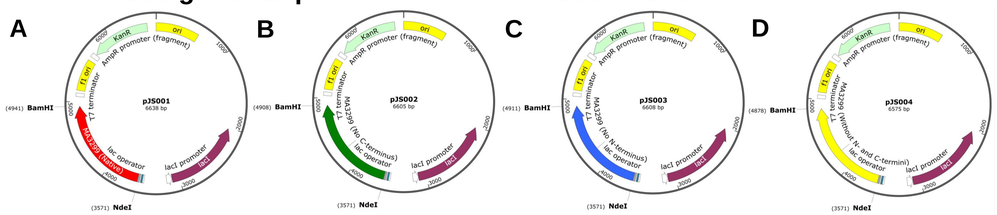

Figure 3. Plasmid maps of *MA3299* variants. A) pJS1, unedited. B) pJS2, without C terminus. C) pJS3, without N terminus. D) pJS4, without N and C termini.

Plasmids Created by Restriction Enzymes and Ligase Procedure

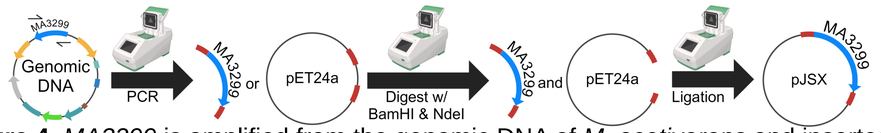

Figure 4. *MA3299* is amplified from the genomic DNA of *M. acetivorans* and inserted into NdeI and BamHI sites in pET24a for inducible expression in *E. coli*. Created with BioRender.com.

Testing Expression of MA3299

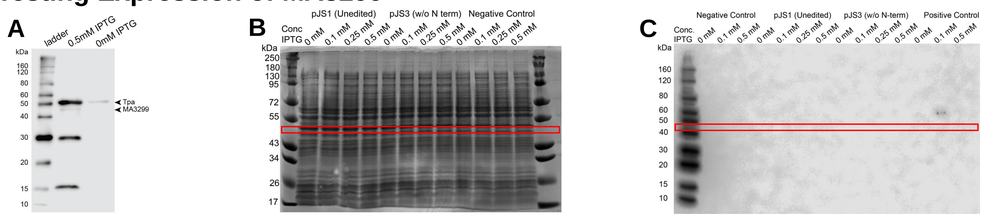

Figure 5. Testing expression of *MA3299* in BL21 *E. coli*. MA3299 protein ~49 kDa. A) Previous Western Blot showing expression is possible. B) Coomassie-stained gel. C) Western Blot 10 minutes of exposure time.

Future Directions

Continuing to attempt *MA3299* expression

- Alter: media composition, IPTG concentration, incubation parameters

Following proper expression, we will:

- Purify using cation exchange chromatography
- Cleave the histidine tag using TEV protease
- Determine the structure using protein crystallography
- Characterize reaction and identify catalytic residues

Acknowledgements

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References

[1]. Thauer, Rudolf K. “The Wolfe cycle comes full circle.” Proceedings of the National Academy of Sciences of the United States of America vol. 109,38 (2012): 15084-5. doi:10.1073/pnas.1213193109.

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