Investigating Effects of Shell Proteins on Molecular Transport in Bacterial Microcompartments

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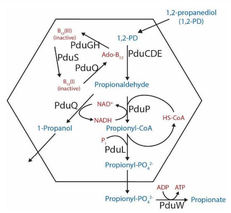
Abstract

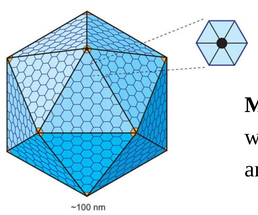
Microcompartments are organelle-like structures that house metabolic reactions within bacteria, functioning to localize enzymes and prevent toxic intermediates from escaping into the cell. The 1,2-propanediol utilization microcompartment (MCP) in Salmonella enterica converts 1,2-propanediol into carbon and energy. The MCP is constructed of shell proteins with pores that let metabolites in and out. Pdu genes control the structure and function of these shell proteins. This research investigates shell protein structure effects on metabolite transport. Previously, mutations to the Pdu A shell protein gene were made by replacing with the Eut M and K26A genes, through the process of recombination. Eut M displayed a superior growth phenotype to WT. I optimized the growth curve assay of Eut M by determining the optimum position in a shaking incubator to decrease margin of error while achieving optimal growth. It was found that Eut M growth was optimized with low light and evenly distributed heat.

Background

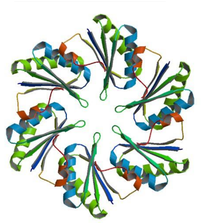
Microcompartments: house reactions, localize enzymes, prevent toxic intermediates from escaping, and serve as a private cofactor pool



Importance: in the future, MCPs can be designed to house nonnative pathways so bacteria can produce commercially important molecules



MCP is made of: shell protein hexamers with pores, which allow metabolites in and out and act as a diffusion barrier **[1]**

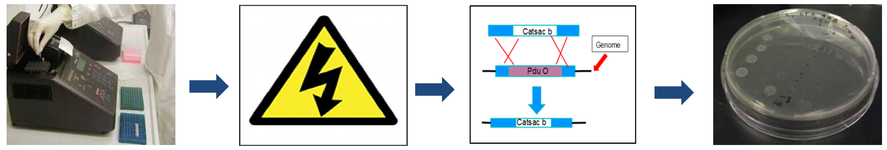


Pdu A: standard pore, possibly involved in substrate and small molecule transport **[2]**

Mutations to Pdu A made-in order to investigate involvement of shell protein structure in metabolite transport

Methods

Recombination: Making Pdu A K26A and ΔPdu A:Eut M strains **[3]**



Polymerase Chain Reaction
amplifies a gene

Transformation
introduces amplified gene by electroporation

Recombination
new gene becomes part of genome

Screen
for cat-sacB cassette

Growth Curve Assays: Comparing Eut M strain to K26A and Wild Type (WT)

Grow cultures
in shaking incubator

Use nanodrop
to measure Optical Density (OD)

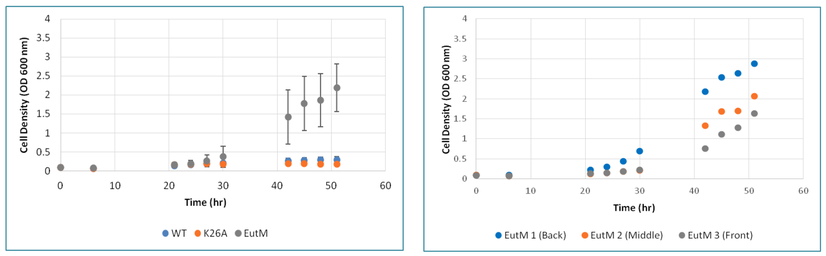
Analyze collected data
to observe growth

Results

Growth Curve 1: Shaking Incubator

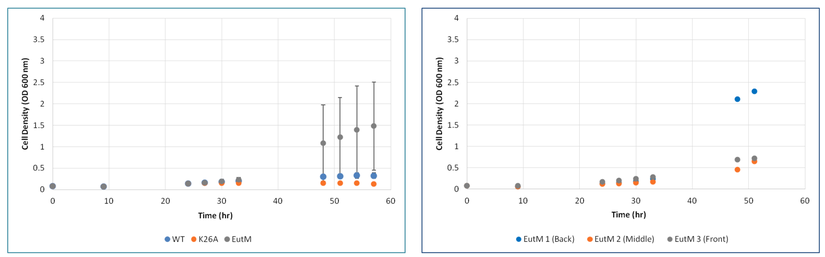
Eut M grew best in back row of the shaker

Significant error was observed



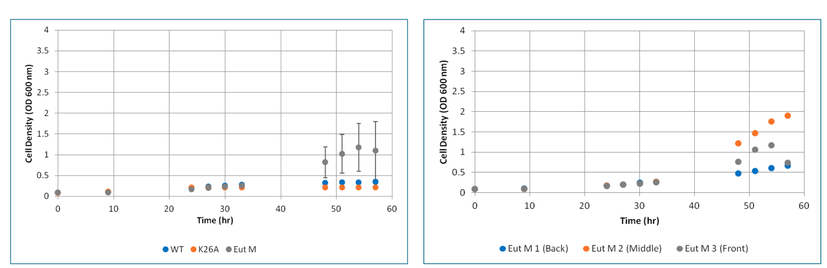
Growth Curve 2: Shaking Incubator with covered window

Eut M again grew best in the back row of the shaker



Growth Curve 3: Water Bath Shaker

Eut M grew best in middle row of water bath shaker



Conclusions

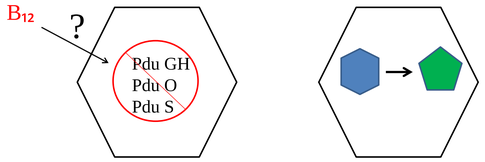
- It was found that the growth curve of Eut M was optimized with minimal light and evenly distributed heat
- In the future, in order for significant data to be collected, several trials of the Eut M growth curve alongside WT and K26A must be run in order to minimize error
- Alternatively, instead of running experiment in triplicate, growth curve may be run with one sample each of Eut M, WT, and K26A in row of shaker that Eut M grew best in

Additional Goals

Use recombination to knockout recycling genes Pdu GH, Pdu O, and Pdu S from 1,2-propanediol MCP to determine if Coenzyme B_{12} diffuses through microcompartment protein shell

Knockouts of Pdu GH and Pdu O currently being made

If it is found that B_{12} diffuses through MCP, mutations to the Pdu B shell protein will be made to observe if pore structure and shape affects B_{12} transport



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2. Crowley, C.S. et. al (2010). Structural insight into the mechanisms of transport across the Salmonella enterica Pdu microcompartment shell. Journal of Biological Chemistry. 285, 37838-37846
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