Engineering Corynebacterium glutamicum to Improve B-Ketoadipate Pathway Productivity

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Aromatic molecules present a sustainable alternative energy to fossil fuels.

- 65 percent of greenhouse gas emissions are from fossil fuels, creating the urgent need for alternatives. [1]
- The government has recently issued an executive order for biomass and bioeconomy helpfulness.

- Lignin is considered a major waste product in biomass production; it can be broken down into different aromatic monomers through chemical pretreatment. [2]
- Microbes are powerful and have diverse applications including: antibiotics, energy, beer, cheese, etc.
- Researchers have used E. coli to break down aromatics in the past, however these molecules are toxic to cells and usually cause death.
- C. glutamicum has natural resistance to the toxicity of these molecules and is generally regarded as safe.
- Beta-ketoadipate, a precursor to nylon-like polymers, can be produced within the aromatic catabolism pathways of C. glutamicum.

Deletion of pcol and pcol Results in Beta-Ketoadipate

- After the deletion of both pcol and pcol our engineered strain, AL23, will no longer be able to metabolize the product, beta-ketoadipate.
- P-Coumarate and Ferulate are both substrates of interest due to their large makeup of lignin.
- C. glutamicum also contains a benzoate pathway that we thought would be interesting to look at.

- A fermentation with 6 g/L of either p-Coumarate or Benzate and 40 g/L of glucose was performed.
- Titers of 2.2 g/L and 2.1 g/L were achieved, respectively. Yields on the other hand were only .37 and .38, respectively.

- Due to low yields, we believed there may be bottlenecks within the pathway, and we found 4-hydroxybenzoate to be the rate-limiting step.

- After feeding the engineered strain 4-hydroxybenzoate, we noticed a significant lag in cell growth.
- If we overexpress poba will we see increased productivity?

Current and Future Work

- Overexpression of genes pcol and pcol to identify a solution for the 4-hydroxybenzoate bottleneck
- 13C Analysis to investigate levulinic acid catabolism in C. glutamicum and other potential pathways were carbons are being lost
- Use lignin hydrolysate for beta-ketoadipate production to demonstrate how this feedstock can be consumed by our engineered strain.

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References