Engineering *Corynebacterium glutamicum* to Improve B-Ketoadipate Pathway Productivity

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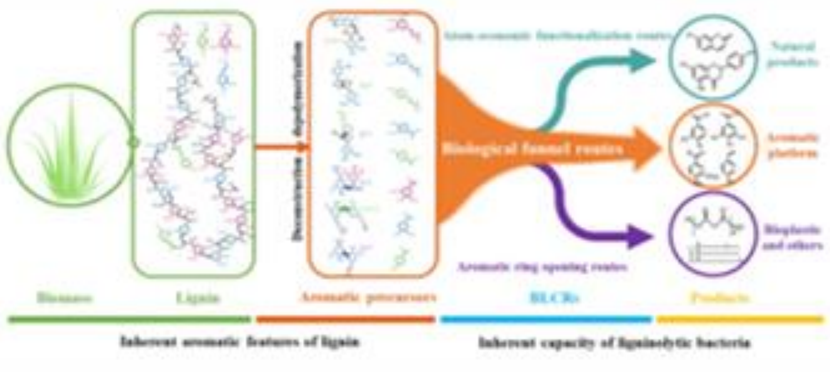
Aromatic molecules present a sustainable alternative energy to fossil fuels.

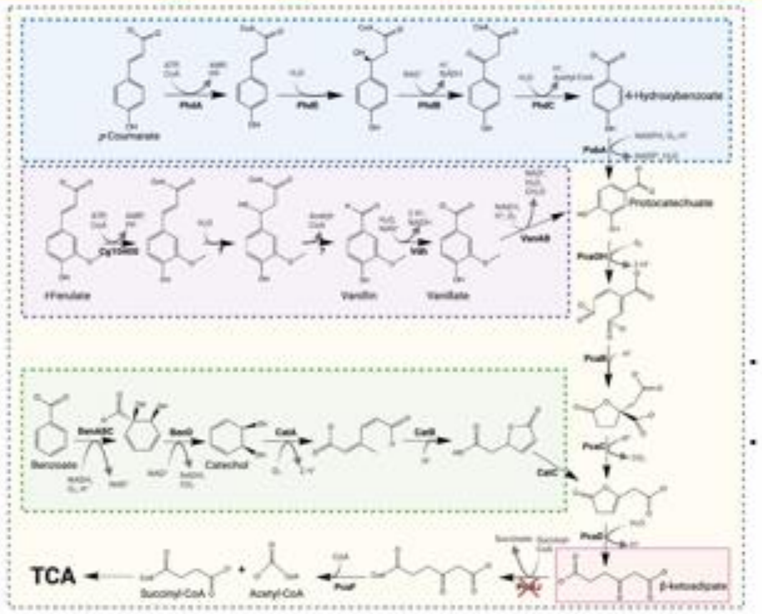
- **65 percent** of greenhouse gas emissions are from fossil fuels, creating the urgent need for alternatives. [1]
- The government has recently issued an **executive order** for biomanufacturing and bioeconomy helpfulness.



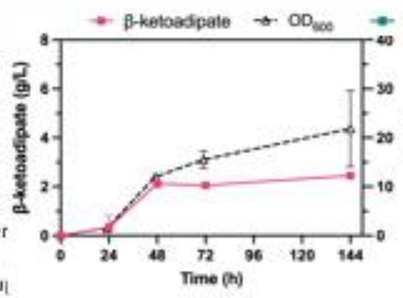
- Lignin is considered a major **waste product** in biomass production; it can be broken down into different **aromatic monomers** through chemical pretreatment. [2]
- Microbes are powerful and have diverse applications including: antibiotics, energy, beer, cheese, etc.
- Researchers have used *E. coli* 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*.

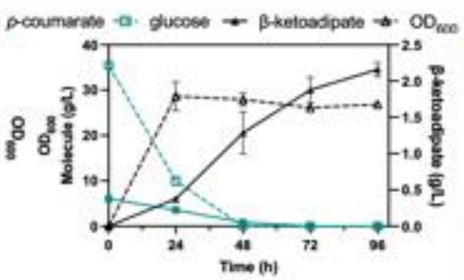
Deletion of *pcaI* and *pcaJ* Results in Beta-Ketoadipate

- After the deletion of both *pcaI* and *pcaJ* 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 Benozate and 40 g/L of glucose was performed.
- Titers of 2.2 g/L and 2.4 g/L were achieved, respectively. Yields on the other hand were only .37 and .38, respectively.





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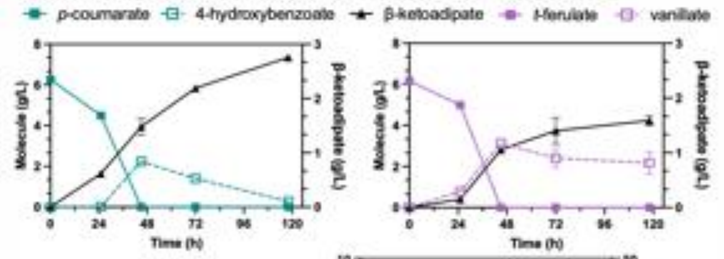
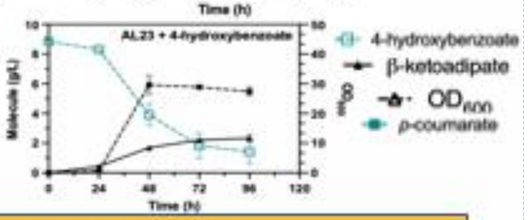
- Due to low yields, we believed there may be bottlenecks within the pathway, and we found 4-hydroxybenzoate to be the rate limiting step.



- 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 *praI* and *pobA* to identify a solution for the 4-hydroxybemzoate bottleneck
- **13-C 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.

Acknowledgements

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References

1) Covert, T., Greenstone, M., & Knittel, C. R. (2016). Will we ever stop using fossil fuels? *The Journal of Economic Perspectives*, *30*(1), 117–138. https://doi.org/10.1257/jep.30.1.117
2) Calvo-Flores, F. G., & Dobado, J. A. (2010). Lignin as renewable raw material. *ChemSusChem (Weinheim. Print)*, *3*(11), 1227–1235. https://doi.org/10.1002/cssc.201000157

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