Improving Succinate Production in *E. coli* through Substrate Channeling

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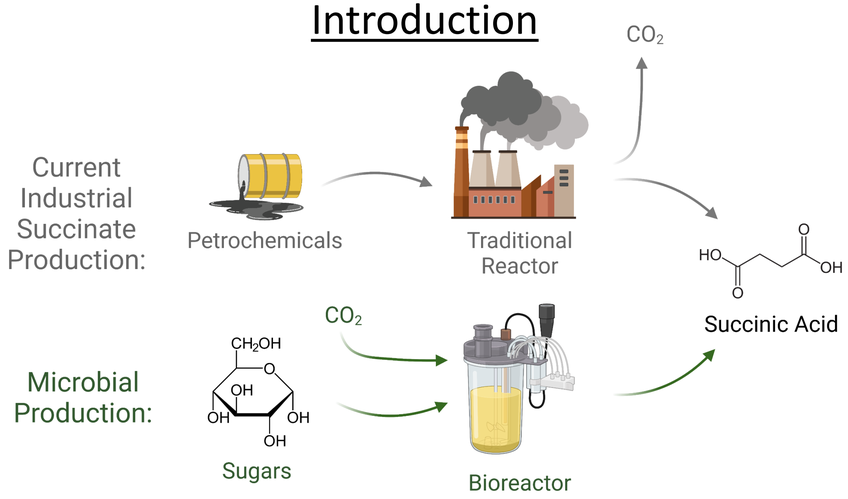
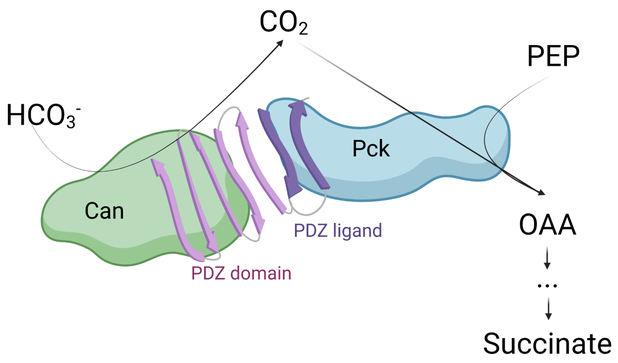
Introduction



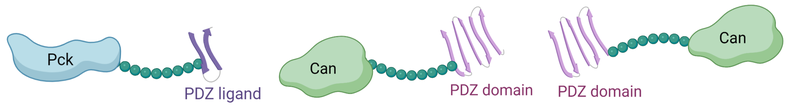
Engineered bacteria provide sustainable routes for succinate production[1]. *E. coli* is potentially limited by its ability to fix CO_2. To improve the local concentration of CO_2 near its carboxylase, **we engineered a new fusion protein.**

Linking Can and Pck

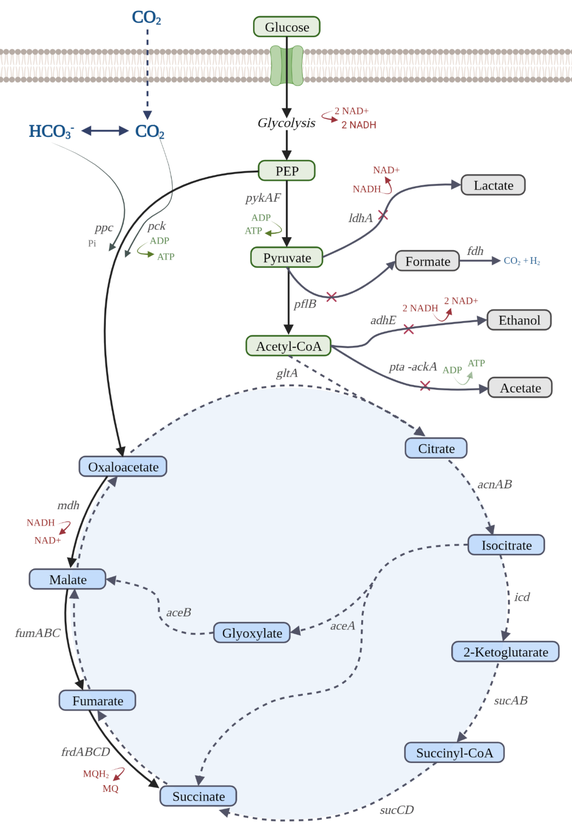


PDZ domains interact and colocalize their attached enzymes.

Plasmids and Proteins Used

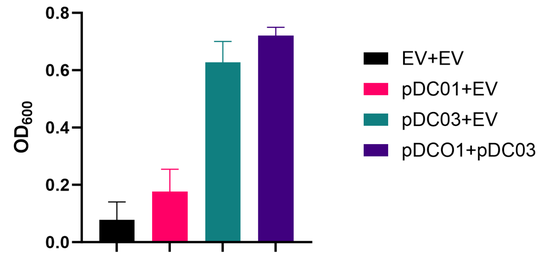


KJ122 Succinate Pathway



Pathway drawn by Amanda Godar in Biorender.

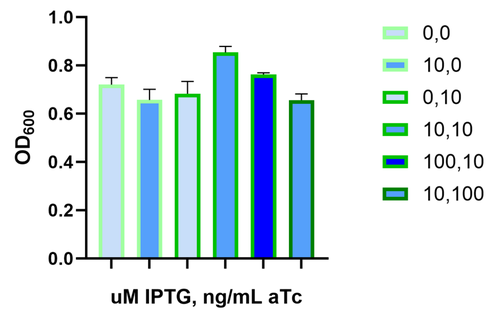
Expressing Fusion Protein



Background strain AG156: Succinate producer KJ122 with can, cynT, and pck deleted.

Conclusion: can is most important for growth restoration.

Optimizing Expression Levels



Conclusion: 10uM IPTG and 10 ng/mL aTc are the optimal inducer concentrations for succinate production.

Future Work

- Testing linked vs. unlinked enzymes
- Testing pDC02 vs. pDC03
- Testing other background strains (with carbonic anhydrase) & other linker methods

FURI

References

1. Ahn, J. H., Jang, Y. S., & Lee, S. Y. (2016). Production of succinic acid by metabolically engineered microorganisms. *Current Opinion in Biotechnology*, 42, 54–66. https://doi.org/10.1016/J.COPBIO.2016.02.034

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