Expression of Non-Native Inorganic Carbon Transporters in E. coli for Improved Succinate Production

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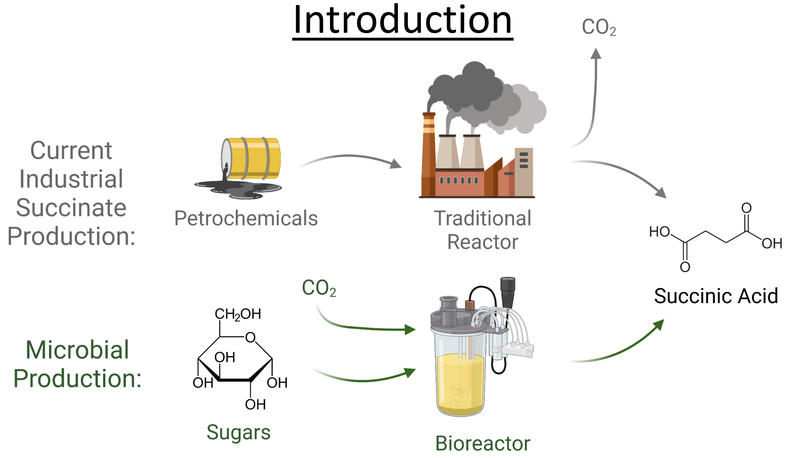
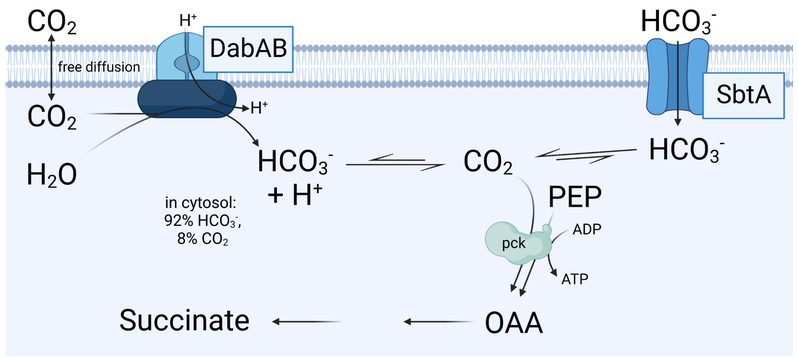
Introduction



Engineered bacteria provide sustainable routes for succinate production[1]. E. coli has no native transporters for active uptake of inorganic carbon. To improve its ability to rapidly import inorganic carbon, **we engineered an *E. coli* strain expressing cyanobacterial bicarbonate transporters.**

The transporters: SbtA and DabAB



While CO_2 can freely diffuse across the cell membrane, HCO_3^- cannot. Thus, SbtA and DabAB can trap inorganic carbon inside the cell in the form of HCO_3^-.

Methods

1. Transformed transporters into chemically competent KJ122 (*dabAB*), carbonic anhydrase-free (Cafree) KJ122 (*sbtA*)
2. Cultured strains overnight in LB media, inoculated at OD 0.1.
3. Fermentations done at 37°C in AM1 minimal media.

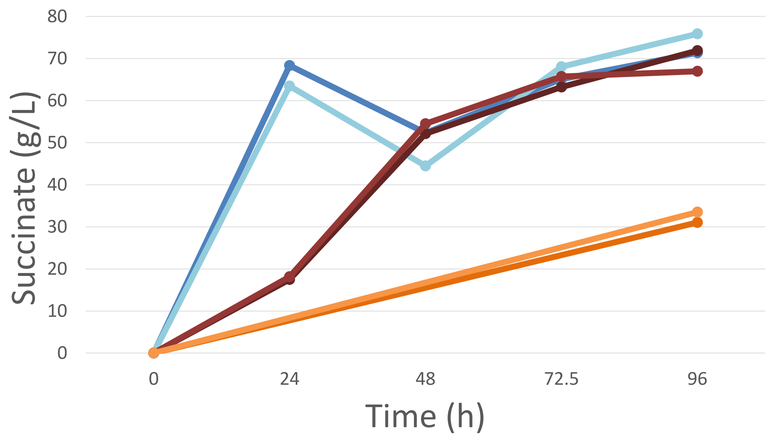
DabAB fermentation

- Control: KJ122 + HCO_3^-
- Tested KJ122 + DabAB (pAG01):
  - Carbon supply: CO_2 from hollow fiber membranes or HCO_3^- in media

SbtA fermentation

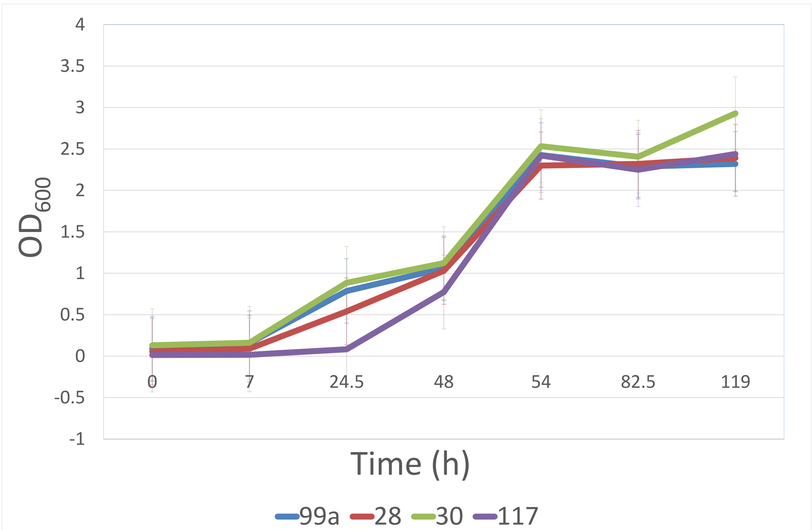
- Control: CAfree KJ122 + empty vector (pTrc99a)
- Tested three Sbta variants in plasmids pSP028, pSP030, pSP117

DabAB Fermentation Results



These results show that the cells with *dabAB* expressed were able to produce succinate titers similar to KJ122.

SbtA Fermentation Results



These results show that the cells with the pSP030 variant grew better than the empty vector control. Since cell growth is linked to succinate production[2], optical density is used as an indicator of succinate production.

Conclusions

SbtA transports HCO_3^- into *E. coli* cells and could increase cytosolic inorganic carbon. The poor growth of the CAfree strains may indicate the necessity for a CA (potentially near Pck). Further testing is needed to confirm.

Future Work

- Inducing *sbtA, dabAB* expression with IPTG
- Synthetic biology strategies to improve K_m and K_{cat} of pck
  - Fusion protein with CA

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
2. Zhang, X., Jantama, K., Moore, J. C., Jarboe, L. R., Shanmugam, K. T., & Ingram, L. O. (2009). Metabolic evolution of energy-conserving pathways for succinate production in Escherichia coli. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(48), 20180–20185. https://doi.org/10.1073/pnas.0905396106

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