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. 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 CO₂ can freely diffuse across the cell membrane, HCO₃⁻ cannot. Thus, SbtA and DabAB can trap inorganic carbon inside the cell in the form of HCO₃⁻.

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 + HCO₃⁻
- Tested KJ122 + DabAB (pAG01):
  - Carbon supply: CO₂ from hollow fiber membranes or HCO₃⁻ 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 the pSP030 variant grew better than the empty vector control. Since cell growth is linked to succinate production, optical density is used as an indicator of succinate production.

SbtA Fermentation Results

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Conclusions

SbtA transports HCO₃⁻ 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 Kₘ and Kₖcat of pck
- Fusion protein with CA

References