Elucidating Growth Coupling of Metabolites in *E. coli* and Characterizing Modularity

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Objective

Project Goal

- The goal of this project is to design a modular (chassis) cell that will increase efficiency of rapid engineering strain design.
- This can be accomplished in a design, build, test, & validation process

Introduction

Background Knowledge

•Metabolism is a systematic network of chemical reactions that drive the transformation of chemical energy to fuel cellular activity[3]

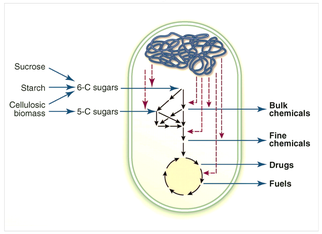


Figure 1: Metabolic Engineering[6]

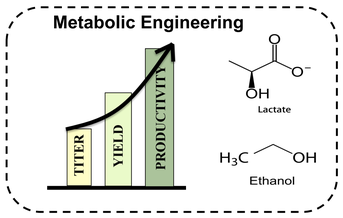


Figure 2: Increased titer, yield, and productivity of desired metabolites

•This would result in optimal economic and sustainable practices of chemical production and synthesis.

Current Limitations

•The current limitation in industrializing biology is the amount of time needed to optimize the production pathways within cells for bulk chemical production.[4]

•Altering DNA elements in a piecewise fashion is a laborious process that the modular cell theory would resolve.[2]

1. The promoter
2. The terminator
3. The copy number
4. The origin of replication

Modularity & Chassis Cell Concept

•Modularity focuses on designing a cellular framework that is auxotrophic, meaning the cell cannot support cellular growth and long term function.[3]

•In theory, the modular cell can be coupled with an engineered production module

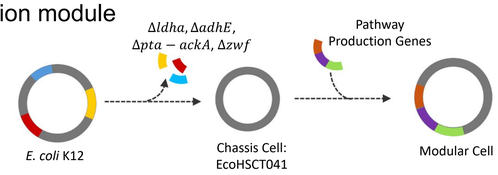


Figure 3: Modular Cell Concept[2]

•This would enhance the production of a target compound and balance redox reactions and metabolic byproducts during the growth of the cell.[3]

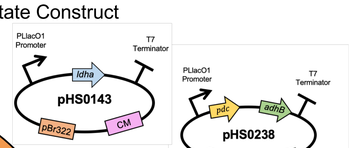
Methodology

- ModCell2 & Multiobjective Strain Design → Modular Cell concept
- Metabolic flux calculations → predict production module products
- PLlacO1 promoter has universal properties in *E. coli*

Step 1: Design

Step 2: Build

Lactate Construct



Ethanol Construct

Step 3: Test

- The growth of cells will be measured using a spectrophotometer to record the turbidity of the culture[1]
- HPLC will be used to measure the yield, productivity and titer of the product.

Step 4: Validate

- Hypothesis: Optimal modular cells will grow up the fastest

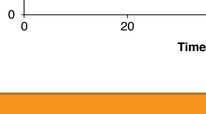


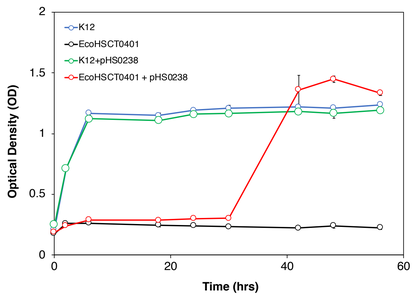
Figure 4: Selection Platform Technique

- Isolate optimal cell strain via serial inoculation[3]

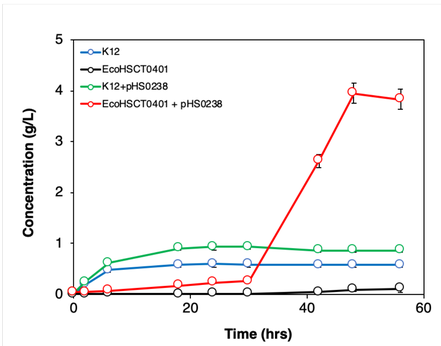
Results

Ethanol

Growth Curve



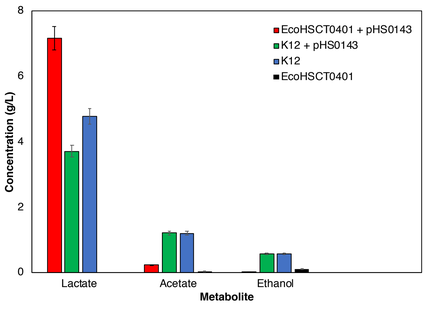
Metabolite Production



Lactate

Growth Curve

Metabolite Production



Discussion

Implications of Experiment Design

K12: Growth possible without Product Synthesis

EcoHSCT0401 + pHS0238: Growth coupled with product synthesis

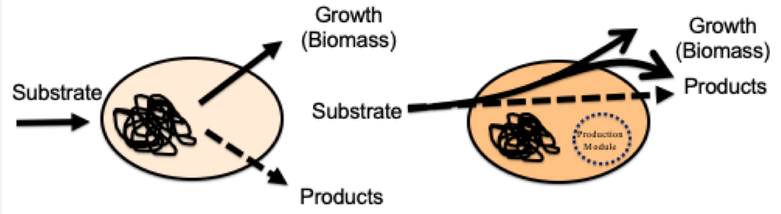


Figure 5: Growth Coupled Overproduction[5]

Future Directions

- Focus on growth coupling of higher order alcohols like isobutanol and butanol
- Investigate other competent cells like BL21 which are engineered for better expression levels.

Mutate Modular Cell → Build Library → Isolate Optimal Modular Cell

Conclusion

- Demonstrating modularity insinuates that there exists a baseline cellular framework from which all types of cellular processes and chemicals can be produced
- This would make chemical synthesis more universal and robust.

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