**Optimizing PHB Production in E. coli Through Metabolic Modeling & Simulation**

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### INTRODUCTION

- **PHB bioproduction:** Expression of PHB synthesis genes in *E. coli* was succeeded by introducing plasmids (Slater, 1988). The PHB synthetic pathway consists of three reactions (Fig1):
  
  **Reaction 1:** β-ketothiolase (PhbA)  
  2 Acetyl-CoA $\rightarrow$ Acetoacetyl-CoA + CoA (1)

  **Reaction 2:** Acetoacetyl-CoA reductase (PhbB)  
  Acetoacetyl-CoA + NADPH + H^+ $\rightarrow$ (R)-3-hydroxybutyryl-CoA + NADP^+ (2)

  **Reaction 3:** PHB polymerase (PhbC)  
  n (R)-3-hydroxybutyryl-CoA $\rightarrow$ Polyhydroxybutyrate + n CoA (3)

- **Flux Balance Analysis (FBA):** FBA calculates the flow rate of metabolites through this metabolic network, thus making it possible to predict the growth rate of an organism and the production rate of an important metabolite.

- **COBRA Toolbox:** An extension tool of MATLAB to implement FBA with genome-scale metabolic model (Orth, 2010).

### OBJECTIVES

- Modeling the metabolism of *E. coli* that produces PHB
- Optimizing the growth media and the genes to maximize PHB production by simulation
- Verification of the simulation results by experiments

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### EXPERIMENTAL RESULTS

- **XL1-Blue** had higher yield of both dry cell and PHB (Fig4).
- **K-12** did not produce PHB.
- MgSO4 and L-glutathione induced PHB production by suppressing cell growth.

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### CONCLUSIONS & FUTURE WORK

- Reduced L-glutathione was advanced as an additional sulfur source by PHB production potential analysis.
- ACK, TPI, and SUCCOAS were introduced as the unwanted reactions by strain design for PHB production.
- Verification of the impact of deletion of the three unwanted reactions will be implemented.

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### EXPERIMENTAL METHODS

- **Strains:** *E. coli* K-12, XL1-Blue
- **Plasmid:** pBHR68 (For PHB production)
- **Media:** 100 mL of M9 media with different Sulfur source (Control, MgSO4, reduced L-glutathione)
- **Measurement of dry cell weight:** Centrifuged the media, freeze the pellet, and dry it
- **Measurement of PHB:** HPLC with frozen pellet

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### REFERENCES


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