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

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SUMMARY

By using the metabolic model of *E. coli* and FBA method, reduced L-glutathione was introduced as an additional sulfur source, and also three unwanted genes were suggested. These simulation results must now be verified experimentally.

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 ↔ Acetoacetyl-CoA + CoA (1)

Reaction 2: Acetoacetyl-CoA reductase (PhbB)

Acetoacetyl-CoA + NADPH + H^+ ↔ (R)-3-hydroxybutyryl-CoA + $NADP^+$ (2)

Reaction 3: PHB polymerase (PhbC)

n (R)-3-hydroxybutyryl-CoA → Polyhydroxybutyrate + n CoA (3)

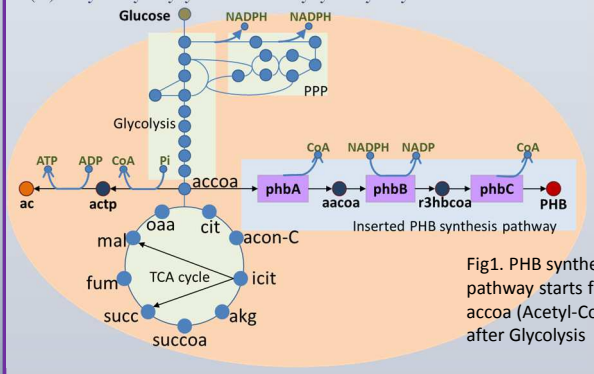

Fig1. PHB synthesis pathway starts from accoa (Acetyl-CoA) after Glycolysis

- **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

SIMULATION METHODS

- **Genome-scale metabolic model:** The employed model is called iJO1366, based on *E. coli* K-12 (Orth, 2011).
- **Production Potential Analysis:** Set the PHB producing reaction as the objective function, and then optimized it with different combinations of nutrients (Feist 2010).
- **Strain Design:** Used three metabolic engineering tools of the COBRA toolbox: OptKnock, OptGene, and GDLS.

SIMULATION RESULTS

Reduced glutathione was suggested as an effective sulfur source for PHB production (Fig2).

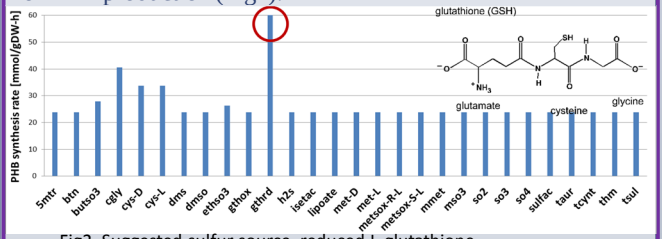

Fig2. Suggested sulfur source, reduced L-glutathione

Also, the simulation showed the unwanted reactions for PHB production (Fig3).

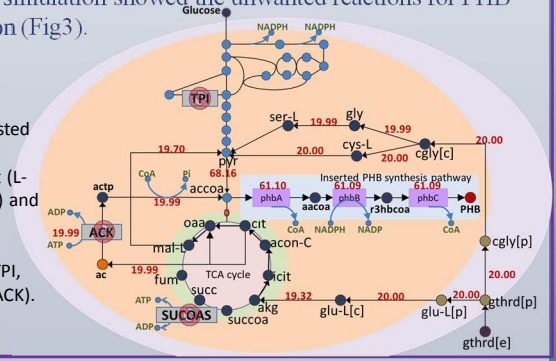

Fig3. Suggested additional supplement (L-glutathione) and suggested unwanted reactions (TPI, SUCCOAS, ACK).

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

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.

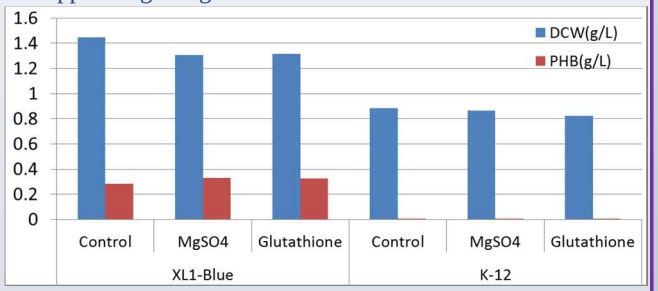

Fig4. Experimental results of different *E. coli* strains (XL1-Blue, K-12) and different sulfur sources (Control, MgSO4, L-glutathione)

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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