Production of 1,3-Propandiol from Glucose by Recombinant Escherichia coli BL21(DE3).

Jaehyeon Lee [1], Suman Lama [1,2], Jungrae Kim[1], Sunghoon Park[2*]

1 Department of Chemical and Biomolecular Engineering, Pusan National University, Busan 46241, South Korea

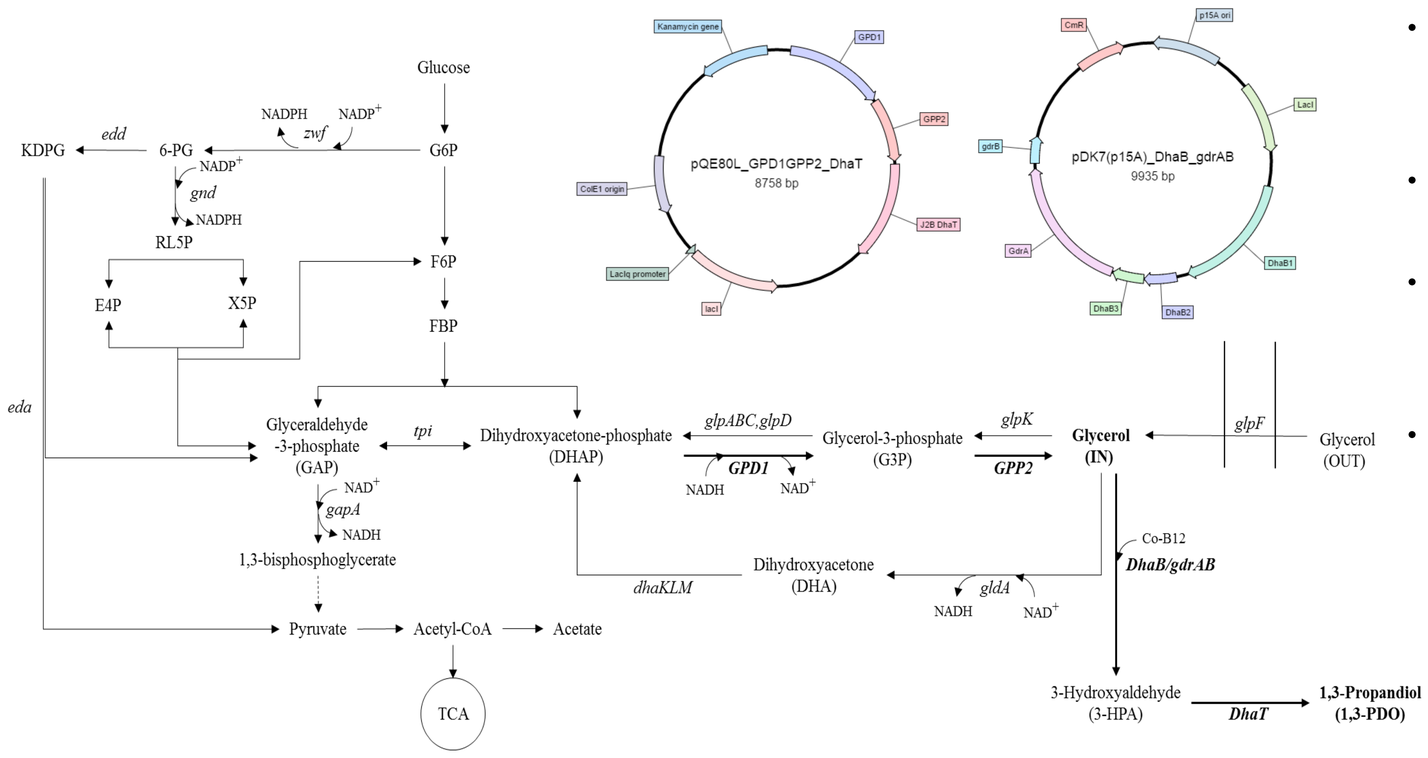
2 School of Energy and Chemical Engineering, Ulsan National Institute of Science and Technology 50 UNIST-gil, Ulju-gun, Ulsan, 44919, South Korea

*parksh@pusan.ac.kr

ABSTRACT

1,3-Propanediol (1,3-PDO) is a commercially valuable platform compound. Its production from glucose by Escherichia coli has been commercialized by DuPont, but no details are not known. In this study, E. coli BL21(DE3) was engineered to have two synthetic pathways, the one converting the glycoytic intermediate dihydroxyacetone phosphate (DHAP) to glycerol; and the other converting glycerol to 1,3-PDO. In addition, the pathway used for assimilatory utilization of glycerol as carbon source was disrupted. Furthermore, to enhance intracellular NAD(P)H availability, the three glucose metabolic pathways (Embden-Meyerhof-Parnas, Pentose phosphate, and Entner-Doudoroff) were modified. The engineered strain could successfully produce 1,3-PDO when grown on glucose. Further study to improve 1,3-PDO yield is under progress.

1,3-PDO production pathway of *E.coli*



Approach

INTRODUCTION

- *E.coli* has been considered as an appropriate host for 1,3-PDO production, because of its well-known metabolism pathway and easy to genetic modification.
- glycerol 3-phosphate dehydrogenase (GPD) and glycerol 3-phosphate phosphatase (GPP) catalyze dihydroxyacetone phosphate to glycerol.
- Glycerol dehydratase (DhaB), a coenzyme B_{12}-dependent enzyme, converts glycerol to 3-hydroxypropionaldehyde (3-HPA), which is intermediate to produce and 1,3-propanediol (1,3-PDO).
- Improving 1,3-PD production and glycerol uptake.
  - External addition of Co-B12 for 1,3-PD production
  - Reducing assimilation of glycerol, glycerol oxidative metabolic pathway gene would be deleted.
  - Improving GlpF expression level for glycerol uptake availability
    - Effect of deletion of ptsG gene
    - Effect of deletion of glpR gene

Effect of external addition of Co-B12

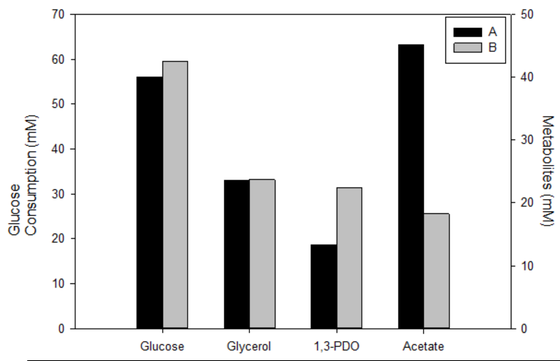


Figure 1:

E.coli BL21(DE3) Δ*glpK*Δ*glpABC* Δ*glpD*,
pQE80L(ColE1) *gpd1 gpp2 dhaT*
pDK7(p15A) *dhaB gdrAB*

A - 0.001mM Co-B_{12}
B - 0.01mM Co-B_{12}

Strains	
EC	*Escherichia coli BL21(DE3)*
EC3	*Escherichia coli BL21(DE3)*) Δ*glpK*Δ*glpABC* Δ*glpD* mutant strain
EC4	*Escherichia coli BL21(DE3)*) Δ*glpK*Δ*glpABC* Δ*glpD* Δ*gldA* mutant strain
EC5	*Escherichia coli BL21(DE3)*) Δ*glpK*Δ*glpABC* Δ*glpD* Δ*ptsG* mutant strain
EC6	*Escherichia coli BL21(DE3)*) Δ*glpK*Δ*glpABC* Δ*glpD* Δ*glpR* mutant strain
Plasmids	
G	pQE80L(ColE1) *gpd1 gpp2 dhaT* pDK7(p15A) *dhaB gdrAB*
B	pDK7(p15A) *dhaB gdrAB*

Table 1. Bacterial strains and plasmids used in this study.

Reduction of glycerol assimilation by deletion of glycerol metabolic pathway

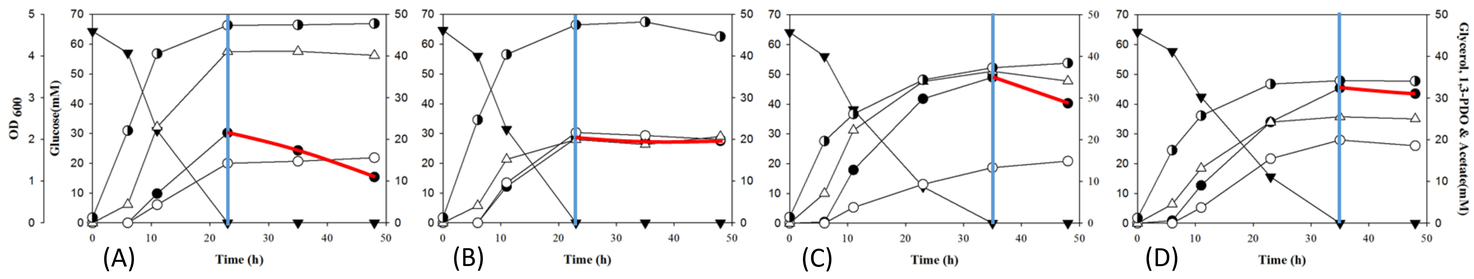


Figure 2: Effect of glycerol assimilation gene Δ*glpK*Δ*glpABC* Δ*glpD* on production of 1,3-PDO. Time course profile of cell growth, glucose consumption, glycerol, 1,3-PDO production and other metabolites production. (A) EC-GB and (B) EC3-GB on aerobic condition. (C) EC-GB and (D) EC3-GB on micro-aerobic condition. Symbols: Cell growth, semi-filled circle; Glucose, closed triangle; Glycerol, closed circle; 1,3-PDO, open circle; acetate, open triangle; open square

Improving GlpF expression level for glycerol uptake availability

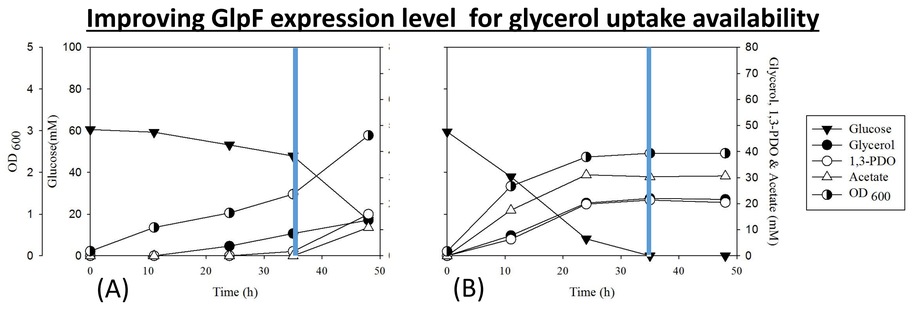


Figure 3: Production of 1,3-PDO by improving GlpF expression for glycerol uptake availability. (A) EC5-GB and (B) EC6-GB in flask scale. The aeration was fixed at 50ml volume media and 250ml flask and the agitation speed was 100 rpm. Symbols: Cell growth, semi-filled circle; Glucose, closed triangle; Glycerol, closed circle; 1,3-PDO, open circle; acetate, open triangle; open square

DISCUSSION

To approach objective, there are some reason of low glycerol uptake, 1) Lack amount of B12 to convert to 1,3-PD 2) CCR problem or repression of glp operon. For 1,3-pdo production, external addition of Co-B12 was conducted and improving glycerol uptake availability, one of glucose transporter chain gene ptsG or glp operon repression gene glpR would be deleted. When almost 10 times higher of B12 was put, 1,3 PD production was increased about 2 times. In addition, acetate production was decreased. when glucose consumption was finished, parent strain glycerol consumption occurred. But, there were not big difference on production and acetate. In case of triple deletion strain there are no glycerol consumption after glucose consumption was finished. it could be said that there are CCR effect to inhibition on working of glpK. Furthermore *ptsG* gene was cut, glucose consumption rate was dramatically decreased and amount of glycerol and 1,3 PD did not change at all. Even though glpR was cut ,amount of glycerol and 1,3 PD did not change at all. Therefor it should be deleted glycerol oxidative pathway gene (glpK, glpD, glpABC). there are no 3-HPA accumulation. so it looks there are lack of availability of NAD(P)H.

ACKNOWLEDGEMENT

This work was supported by the Advanced Biomass R&D Center(ABC) of Global Frontier Project funded by the Ministry of Science, ICT and Future Planning (ABC-2011-0031361)

REFERENCE

1) Deckwer, W.D., 1995. Microbial conversion of glycerol to 1,3-propanediol. FEMS Microbiol. Rev. 16, 143–.149.

2) Andrea, K.F., Rocio, S., Mark, T.H., 2010. Effect of biodiesel glycerol type and fermentor configuration on mixed-acid fermentations. Bioresour. Technol. 101, 9185–.9189.

3) Tong, I-Teh, Hans H. Liao, and Douglas C. Cameron. "1, 3-Propanediol production by Escherichia coli expressing genes from the Klebsiella pneumoniae dha regulon." Applied and environmental microbiology 57.12 (1991): 3541-3546.