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Effective production of *n*-butanol in *Escherichia coli* utilizing the glucose–glycerol mixture

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ABSTRACT

Glucose from plant cellulose and glycerol from the waste stream of the biodiesel production process are two most abundant and renewable carbon sources. *n*-Butanol is an alternative fuel of potential and its production by microbes which utilize the two carbons appears to be industrially incentive on the ground of sustainability and economy. However, the hierarchical regulation of glucose dictates *Escherichia coli* to prefer utilization of glucose over others. This physiological behavior of the cell prolongs the fermentation process and eventually reduces productivity. In this study, a single strain capable of co-utilizing glucose and glycerol was developed by metabolic engineering. Meanwhile, a dual-culture system was also designed by construction of a glucose-selecting and a glycerol-selecting strain. As a consequence, both approaches result in the strains for effective co-utilization of glucose and glycerol. In particular, the single strain enabled production of 6.2 g/L *n*-butanol, which leads to the conversion yield and productivity accounting for 76.5% of the theoretical and 0.17 g/L/h, respectively. It indicates a promise of the developed technology platform for microbial production of *n*-butanol from the glucose–glycerol mixture.

1. Introduction

n-Butanol displays a better property than ethanol in terms of energy density, volatility, and hygroscopicity and is recognized as an alternative fuel of potential [1]. In particular, this chemical enables to fuel vehicle motors after mixing with any concentration of gasoline and can be transported with existing infrastructure [2]. It has been historically produced in *Clostridium* species by the acetone–butanol–ethanol (ABE) fermentation process [3]. Although commercially available, the production efficiency of the ABE bioprocess still needs to improve. Thus far, many strategies have been proposed to address this issue. The microbial production of *n*-butanol from the renewable feedstock appears to be appealing because it provides a sustainable and environment-friendly fuel as crude oil replacement. This helps to ameliorate the global warming effect caused by the enormous emission of greenhouse gas and to constantly provide a stable energy source for the future need [4].

Glucose and glycerol are two renewable carbon sources and particularly useful for microbial fermentation. The former sugar comes from cellulose which is the main component of lignocellulose derived from plant cell walls, the most abundant materials in nature. Therefore, it is incentive to produce biofuels and chemical commodities by microbes using cellulose hydrolysate. Biodiesel is another green transportation fuel of commercial importance, and the extending market demand has substantially increased its annual production yield. It leads to the availability of a large volume of glycerol which is generated in the waste stream of the biodiesel production process [5]. The surplus and the reduced nature of glycerol makes it an excellent sugar for the fermentative production of value-added products by microbes, consequently favoring the economic viability of the glycerol-based industry [6,7].

The fermentative production of *n*-butanol can be achieved in surrogate microbes that harbor the clostridial CoA-dependent synthetic pathway for *n*-butanol [8–11]. Mainly conducted with glucose, these research efforts are afflicted by a low production titer. This is ascribed to the insufficient output of NADH from glucose catabolism to fulfill the reductive need for the synthesis of *n*-butanol. Known as the workhorse of biotechnology, *E. coli* has been successfully engineered to produce a high level of *n*-butanol by recruitment of NADH-generation reaction steps [12–14]. In

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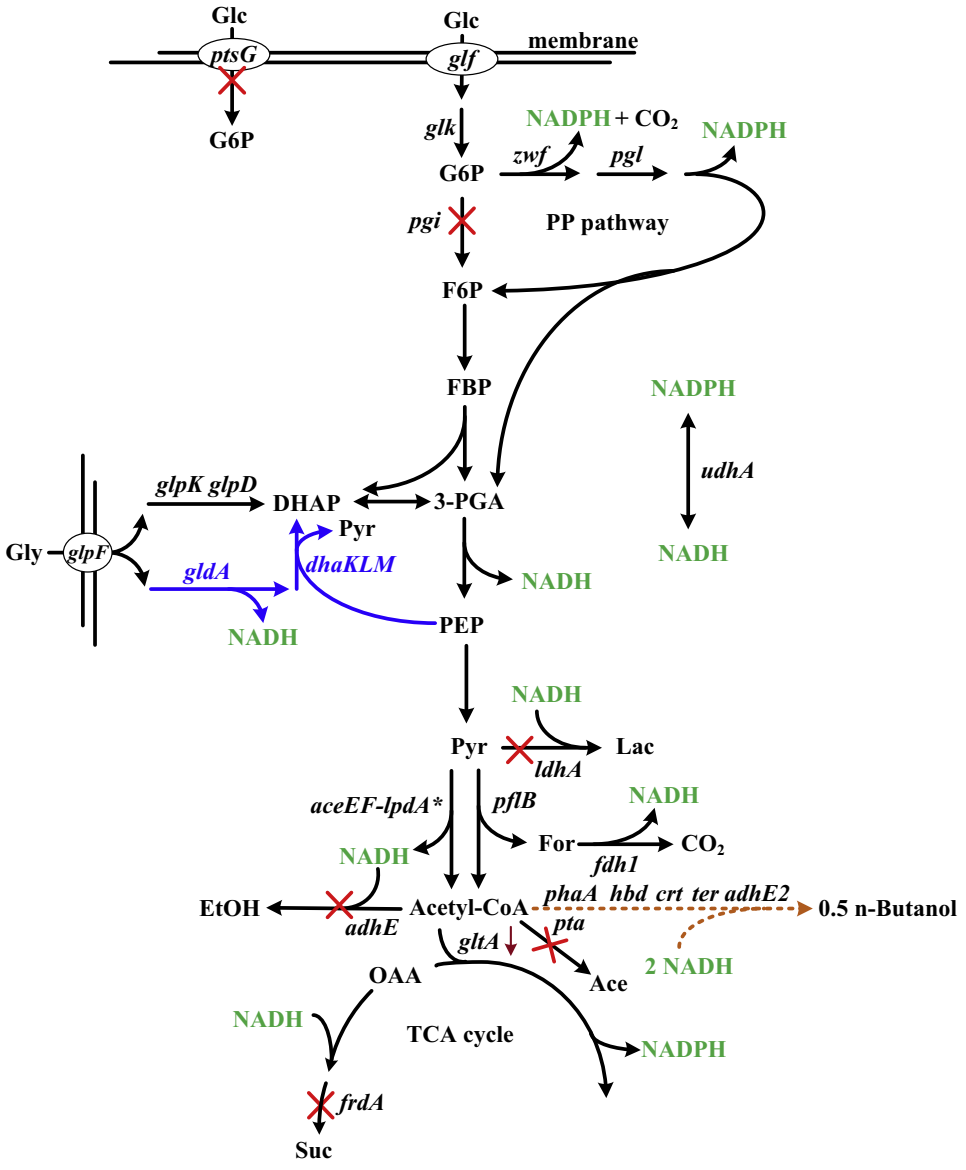

Fig. 1. The central metabolic pathways leading to the *n*-butanol synthesis in *E. coli* strain BuT-14. The genes involved in the metabolic pathways: *aceEF-lpdA**: pyruvate dehydrogenase complex; *adhE*, aldehyde-alcohol dehydrogenase; *adhE2*, butyraldehyde-butanol dehydrogenase; *crt*, crotonse; *hbd*, 3-hydroxybutyryl-CoA dehydrogenase; *ldhA*, lactate dehydrogenase; *fdh1*, formate dehydrogenase; *frdA*, subunit of fumarate reductase; *glk*, glucokinase; *gltA*, citrate synthase; *glpF*, glycerol facilitator; *gldA*, glycerol dehydrogenase; *dhaKLM*, dihydroxyacetone kinase; *glpK*, glycerol kinase; *glpD*, glycerol 3-phosphate dehydrogenase; *pflB*, pyruvate-formate lyase; *pgi*, phosphoglucose isomerase; *pgl*, lactonase; *phaA*, acetoacetyl-CoA thiolase; *pta*, phosphate acetyltransferase; *ptsG*, glucose transporter; *ter*, trans-enoyl-CoA reductase; *udhA*, transhydrogenase; *zwf*, glucose-6-phosphate dehydrogenase. The deleted genes are indicated by "X". Abbreviations: Ace, acetate; DHAP, dihydroxyacetone phosphate; EtOH, ethanol; F6P, fructose-6-phosphate; Lac, lactate; For, formate; G6P, glucose-6-phosphate; FBP, fructose 1,6-bisphosphate; Glc, glucose; Gly, glycerol; OAA, oxaloacetate; PEP, phosphoenolpyruvate; 3-PGA, 3-phosphoglyceraldehyde; Pyr, pyruvate; Suc, succinate.

contrast, we proposed a novel production platform consisting of two *E. coli* strains that builds up a redox-balanced synthetic pathway for *n*-butanol [15]. In addition, we rewired the fueling pathways involving glycolysis, the pentose phosphate (PP) pathway, and the tricarboxylic acid (TCA) cycle to increase the intracellular NADH level [16]. Consequently with glucose, these two approaches enable *E. coli* to produce *n*-butanol in an effective way.

To render the *n*-butanol bioprocess appealing relies in the use of cost-effective feedstock. Apparently, it is incentive to produce *n*-butanol in *E. coli* that co-utilizes glucose and glycerol. In *E. coli*, the glucose-specific permease $EIICB^{glc}$ (encoded by *ptsG*) in the phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS) is responsible for glucose uptake. During the transport of glucose, $EIIA^{Glc}$ (encoded by *crr*) is dephosphorylated and fails to stimulate the activity of adenylate cyclase. This in turn lowers the level of cyclic AMP (cAMP), which makes the protein complex consisting of cAMP bound to catabolite activator protein (cAMP-CAP) less available. The expression of the cAMP-CAP complex-dependent genes is consequently repressed, a regulatory mechanism known as catabolite repression [17]. Inducer exclusion is another glucose-exerted regulation mode that mediates the inhibition of certain non-PTS sugar transporters by the non-phosphorylated $EIIA^{Glc}$ to prevent the entry of these sugars [17]. The aerobic catabolism of glycerol in *E. coli* involves *glpF*, *glpK*, and *glpD* (Fig. 1). The expression of *glpFK* and *glpD* is subject to catabolite repression while the GlpK activity is inhibited by the non-phosphorylated $EIIA^{Glc}$. Accordingly, glycerol is not utilized by *E. coli* in the presence of glucose. This diauxic utilization pattern of mixed sugars prolongs the fermentation process and reduces productivity.

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