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Original Research Article

Metabolic engineering of *Corynebacterium glutamicum* for the production of 3-hydroxypropionic acid from glucose and xylose

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ABSTRACT

3-Hydroxypropionic acid (3-HP) is a promising platform chemical which can be used for the production of various value-added chemicals. In this study,*Corynebacterium glutamicum* was metabolically engineered to efficiently produce 3-HP from glucose and xylose via the glycerol pathway. A functional 3-HP synthesis pathway was engineered through a combination of genes involved in glycerol synthesis (fusion of *gpd* and *gpp* from *Saccharomyces cerevisiae*) and 3-HP production (*pduCDEGH* from *Klebsiella pneumoniae* and aldehyde dehydrogenases from various resources). High 3-HP yield was achieved by screening of active aldehyde dehydrogenases and by minimizing byproduct synthesis ($gapA^{A1G}$Δ*ldhA*Δ*pta-ackA*Δ*poxB*Δ*glpK*). Substitution of phosphoenolpyruvate-dependent glucose uptake system (PTS) by inositol permeases (*iolT1*) and glucokinase (*glk*) further increased 3-HP production to 38.6 g/L, with the yield of 0.48 g/g glucose. To broaden its substrate spectrum, the engineered strain was modified to incorporate the pentose transport gene *araE* and xylose catabolic gene *xylAB*, allowing for the simultaneous utilization of glucose and xylose. Combination of these genetic manipulations resulted in an engineered *C. glutamicum* strain capable of producing 62.6 g/L 3-HP at a yield of 0.51 g/g glucose in fed-batch fermentation. To the best of our knowledge, this is the highest titer and yield of 3-HP from sugar. This is also the first report for the production of 3-HP from xylose, opening the way toward 3-HP production from abundant lignocellulosic feedstocks.

1. Introduction

3-Hydroxypropionic acid (3-HP) is a promising platform chemical which is considered as one of the top 12 value added chemicals from biomass by US Department of Energy (Werpy and Petersen, 2004). 3-HP can be used as a renewable building block to produce a wide range of chemicals, including acrylic acid, acrylamide, malonic acid, and 1,3-propanediol (Chen and Liu, 2016; Kumar et al., 2013). Among these derivatives, acrylic acid is the most promising with a global market of 4.6 million tons. Biological production of 3-HP from cheap and renewable feedstock has received significant attention in recent years. Large efforts have been made to develop efficient recombinant strains, especially *Escherichia coli* (Chu et al., 2015; Lim et al., 2016; Liu et al., 2016; Song et al., 2016; Tokuyama et al., 2014) and *Klebsiella pneumoniae* (Ashok et al., 2013a, 2013b; Huang et al., 2013), to produce 3-HP either from glucose or glycerol. Recently, *Saccharomyces cerevisiae* (Borodina et al., 2014; Chen et al., 2014a, 2014b) and *Synechococcus elongatus* (Lan et al., 2015) were also evaluated as potential platforms for 3-HP production. However, the titer (< 80 g/L), yield (< 0.5 g/g) and productivity (< 1.8 g/L/h) that so far has been achieved is far from the requirement of 3-HP industrialization. To be competitive to the petrochemical acrylic acid, the yield and productivity of 3-HP should be significantly improved.

Corynebacterium glutamicum is a gram-positive bacterium which has been widely used for amino acids production in industry (Bommareddy et al., 2014; Chen et al., 2013, 2015b, 2014a, 2014b; Geng et al., 2013; Hasegawa et al., 2013). Metabolic engineering and protein engineering have been intensively used to improve its production capacity, and to enlarge its substrate and product spectrum (Chen and Zeng, 2016, 2013). The titer of glutamate and lysine by *C. glutamicum* can reach as high as 150 g/L in commercial scale (Becker and Wittmann, 2012). *C. glutamicum* is also highly effective for the production of other organic acids, including lactate and succinate with titers of 130–200 g/L (Tsuge et al., 2015). Recently, applications of this microorganism have been extended for the production of other chemicals, such as isobutanol (Blombach et al., 2011), cadaverine (Buschke et al., 2011), and ethylene glycol (Chen et al., 2016). Due to its wide substrate spectrum and high capacity for organic acid production, *C. glutamicum* can be considered as a high-potential workhorse for 3-HP production.

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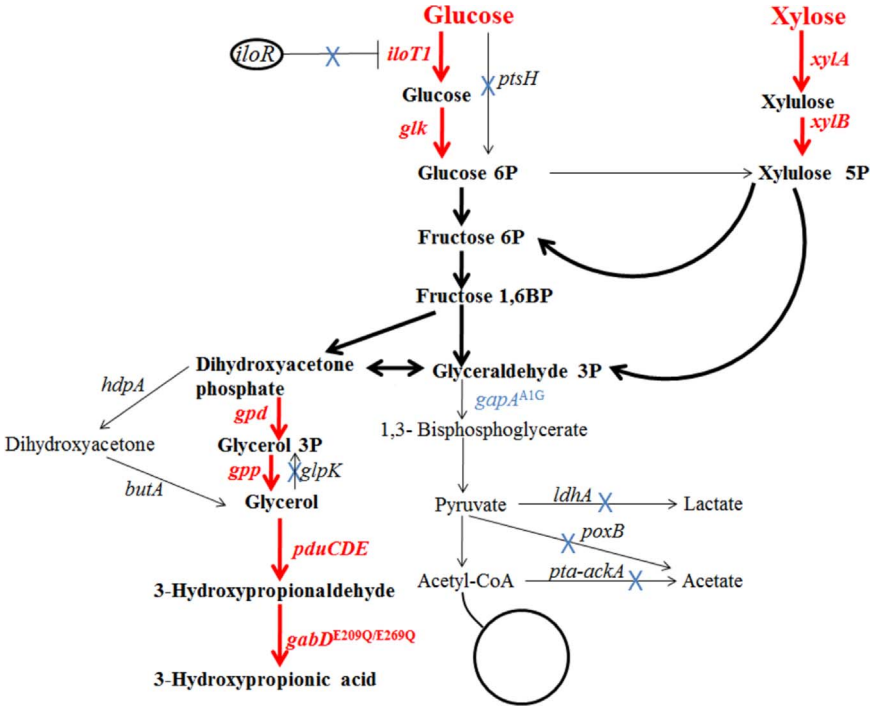

Fig. 1. Reconstruction of the biosynthetic route for 3-hydroxypropionic acid (3-HP) production in *Corynebacterium glutamicum*. Deleted genes are indicated with a cross. Red arrows represent overexpressed genes. Bold arrows indicate high metabolic fluxes necessary for 3-HP overproduction. The glyceraldehyde 3-phosphate dehydrogenase gene (*gapA*) was downregulated by the substitution of the start codon from ATG to GTG. Genes and coded enzymes: *iolT1*, myo-inositol permease; *glk*, glucokinase; *iloR*, transcriptional regulator of myo-inositol utilization genes; *xylA*, xylose isomerase; *xylB*, xylulokinase; *gpd*, glycerol 3-phosphate dehydrogenase; *gpp*, glycerol 3-phosphate phosphatase; *glpK*, glycerol kinase; *pduCDE*, diol dehydrogenase; *gabD*$^{E209Q/E269Q}$, aldehyde dehydrogenase with the point mutation of E209Q/E269Q; *hdpA*, HAD superfamily phosphatase; *butA*, 2,3-butanediol dehydrogenase; *gapA*, glyceraldehyde 3-phosphate dehydrogenase; *ldhA*, lactate dehydrogenase; *poxB*, pyruvate oxidase; *pta*, phosphate acetyltransferase; *ackA*, acetate kinase; *ptsH*, HPr of the sugar: phosphoenolpyruvate phosphotransferase system.

In this study, we attempted to engineer *C. glutamicum* for efficient 3-HP production from lignocellulosic sugars, especially with the most abundant glucose and xylose. Different metabolic pathways have been predicted and used for the production of 3-HP from several intermediates including glycerol, lactate, malonyl-CoA, and β-alanine (Chu et al., 2015; Lim et al., 2016; Liu et al., 2016; Song et al., 2016; Tokuyama et al., 2014). The lactate pathway is thermodynamically unfavorable and thus inefficient for 3-HP production. The malonyl-CoA and β-alanine pathways have been intensively studied, however, the titers of the engineered strains are still low due to complex metabolic regulation (Liu et al., 2016; Song et al., 2016). The glycerol metabolic pathway is the shortest pathway toward 3-HP production from glucose. A similar pathway has been successfully implemented in *E. coli* for commercial 1,3-propanediol production by Dupont. Production of 3-HP from glucose via glycerol is a redox-neutral process with the theoretical yield close to 2.0 mol/mol. For glycerol pathway, 3-HP is synthesized from glycerol by glycerol dehydratase and aldehyde dehydrogenase (Fig. 1). In this study, we reconstructed the glycerol and 3-HP synthesis pathway in *C. glutamicum*. By enzyme screening and enforcement of the flux toward 3-HP synthesis pathway, we demonstrated that *C. glutamicum* was effective for 3-HP production with high titer (62.6 g/L) and yield (0.51 g/g glucose). Downregulation of glyceraldehyde 3-phosphate dehydrogenase and employment of the non-PTS glucose-uptake system are important for increasing the yield of 3-HP. We also successfully engineered *C. glutamicum* to simultaneously utilize glucose and xylose for 3-HP production, opening the door toward efficient production of 3-HP from lignocellulose feedstock.

2. Materials and methods

2.1. Bacterial strains and plasmids

Strains and plasmids used in this study are listed in Table 1. *E. coli* DH5α was used for routine cloning procedures. *C. glutamicum* MB001, a prophage-free strain derived from *C. glutamicum* ATCC 13032, was used as a background strain (Baumgart et al., 2013). The suicide vector pK18mob*sacB* was used for markerless gene deletion, insertion, and substitution by double crossover recombination as described before (Chen et al., 2016). *E. coli/C. glutamicum* shuttle vectors pEC-K18mob2 (Tauch et al., 2002) and pXMJ19 (Jakoby et al., 1999) were used for gene overexpression.

2.2. Plasmids and strains construction

Genes encoding glycerol 3-phosphate dehydrogenase (GPD1) and glycerol 3-phosphate phosphatase (GPP2) from *S. cerevisiae* were codon-optimized and synthesized as an operon with a strong constitutive promoter H30 (Yim et al., 2013). The fragment was cloned into the restriction site of EcoRI/XbaI of pEC-K18mob2, generating pEC-*gpd-gpp*. Similarly, the gene encoding fused GPD1-GPP2 protein, which contains a linker of VVIW between I383 of GPD1 and G2 of GPP2, was synthesized with promoter H30 and cloned into the restriction site of EcoRI/XbaI of pEC-K18mob2, yielding pEC-fusion-*gpd-gpp*. The aldehyde dehydrogenase (GabD) from *Cupriavidus necator* (UniProt accession no. Q0JZI5) with a double point mutation of E209Q/E269Q was also codon-optimized and synthesized (Chu et al., 2015). The nucleic acid sequences and amino acid sequences of the synthesized proteins are listed in Supplementary Figure 1–3.

The *pduCDEGH* gene encoding diol dehydratase and its activator was amplified from the genome of *K. pneumoniae* DSM 2026 using primer 11-F/12-R and inserted into the restriction site of EcoRI/KpnI of pXMJ19, giving pXMJ-*pduCDEGH* (Gibson et al., 2009). The mutant *gabD* gene, *aldH* gene, and *puuC* gene were amplified from the synthesized *gabD* fragment, the genome of *E. coli* K12 and *K. pneumoniae* DSM 2026 using primer pairs of 13-F/14-R, 15-F/16-R and 17-F/18-R, respectively. The corresponding fragments were in-

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