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Metabolic engineering of *Escherichia coli* for the production of 5-aminovalerate and glutarate as C5 platform chemicals

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ABSTRACT

5-Aminovalerate (5AVA) is the precursor of valerolactam, a potential building block for producing nylon 5, and is a C5 platform chemical for synthesizing 5-hydroxyvalerate, glutarate, and 1,5-pentanediol. *Escherichia coli* was metabolically engineered for the production of 5-aminovalerate (5AVA) and glutarate. When the recombinant *E. coli* WL3110 strain expressing the *Pseudomonas putida davAB* genes encoding delta-aminovaleramidase and lysine 2-monooxygenase, respectively, were cultured in a medium containing 20 g/L of glucose and 10 g/L of L-lysine, 3.6 g/L of 5AVA was produced by converting 7 g/L of L-lysine. When the *davAB* genes were introduced into recombinant *E. coli* strainXQ56allowing enhanced L-lysine synthesis, 0.27 and 0.5 g/L of 5AVA were produced directly from glucose by batch and fed-batch cultures, respectively. Further conversion of 5AVA into glutarate could be demonstrated by expression of the *P. putida gabTD* genes encoding 5AVA aminotransferase and glutarate semialdehyde dehydrogenase. When recombinant *E. coli* WL3110 strain expressing the *davAB* and *gabTD* genes was cultured in a medium containing 20 g/L glucose, 10 g/L L-lysine and 10 g/L α-ketoglutarate, 1.7 g/L of glutarate was produced.

1. Introduction

As our concerns on the environmental problems and fossil resource availability increase, there has been much interest in producing chemicals and materials from renewable biomass through biorefineries. Several bioprocesses have been successfully developed to produce chemicals, fuels, and polymers from renewable resources by employing metabolically engineered microorganisms. Several platform bacteria such as *Escherichia coli*, *Corynebacterium glutamicum*, and *Lactobacillus* have been metabolically engineered to produce alcohols, polymers, and platform chemicals, including isopropanol (Hanai et al., 2007), butanol (Atsumi et al., 2008a, 2008b; Atsumi and Liao, 2008; Shen and Liao, 2008; Berezina et al., 2010), isobutanol (Smith et al., 2010), higher alcohols (Zhang et al., 2008), diamines (Qian et al., 2009, 2011), 3-hydroxypropionate (3HP) (Rathnasingh et al., 2009), lactic acid (Nguyen et al., 2012), succinic acid (Hong et al., 2004), polylactic acid (PLA) (Jung et al., 2010; Yang et al., 2010, 2011), and polyhydroxyalkanoates (Park et al., 2012a). Among these, 3HP, lactic acid, and succinic acid are of great importance for industrial applications, as they can be used as building-block chemicals for value-added chemical derivatives and for polymer synthesis.

5-Aminovalerate (5AVA) is the precursor of valerolactam, a potential building block for producing nylon 5, and can potentially be used as a C5 platform chemical for synthesizing 5-hydroxyvalerate, glutarate, and 1,5-pentanediol. 5AVA is a metabolite of L-lysine catabolism in *Pseudomonas putida*, in which L-lysine is converted to glutarate through the so-called aminovalerate pathway via 5-aminovaleramide, 5AVA, and glutarate semialdehyde (Revelles et al., 2005, 2007). Glutarate is then channeled into the Krebs cycle via glutaryl-CoA and acetyl-CoA. 5AVA synthesis from L-lysine is mediated by lysine 2-monooxygenase and delta-aminovaleramidase encoded by the *davB* and *davA* genes, respectively, in *P. putida* (Revelles et al., 2005, 2007), but 5AVA production has not been extensively examined in microorganisms.

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The accumulation of a small amount of 5AVA is detected by deleting the genes involved in L-lysine catabolism such as *davT*, *davB*, and *davA* in *P. putida* (Revelles et al., 2005, 2007). Enzymatic synthesis of 5AVA has been developed by employing L-lysine α-oxidase from the fungus *Trichoderma viride*, in which L-lysine is converted to 6-amino-2-ketocaproic acid by releasing H_2O_2, and then H_2O_2 mediates oxidative decarboxylation of 6-amino-2-ketocaproic acid into 5AVA (Pukin et al., 2010). However, direct fermentative production of 5AVA has not yet been demonstrated.

In this study, we report metabolic engineering of *E. coli* for the production of 5AVA from L-lysine by establishing a pathway composed of the *P. putida davB* and *davA* genes encoding lysine 2-monooxygenase and delta-aminovaleramidase, respectively (Fig. 1). The metabolic pathway for producing glutarate from L-lysine was also constructed by expressing the *P. putida gabTD* and *davAB* genes in recombinant *E. coli* (Fig. 1). Furthermore,5AVA and glutarate production from glucose without feeding L-lysine was examined in metabolically engineered *E. coli* that can biosynthesize L-lysine, the major precursor of 5AVA.

2. Materials and methods

2.1. Bacterial strains, plasmids, and genes

All bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* XL1-Blue (Stratagene Cloning Systems, La Jolla, CA, USA) was used for general gene cloning studies. Recombinant *E. coli* WL3110 (Park et al., 2007) and *E. coli* XQ56 (Qian et al., 2011) strains were used as host strains for producing5AVA and glutaric acid. The pKE12-MCS and pKA32-MCS plasmids are expression vectors containing the $P_{LlacO-1}$ promoter (Park et al., 2012b), and The pTacLacI plasmid is the expression vector containing the *tac* promoter (Lee et al., 2008).

2.2. Plasmid construction

All DNA manipulations were performed following standard procedures. The polymerase chain reaction (PCR) was performed with the C1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA). The primers used in this study (Table 2) were synthesized by Bioneer (Daejeon, Korea). The pKE112-MCS plasmid was constructed by replacing the $P_{LlacO-1}$ promoter in pKE12-MCS with the *tac* promoter amplified from pTacLacI at the *Xho*I/*Eco*RI sites. pKE112-DavAB was constructed by sequential insertion of synthesized *P. putida davA* and *davB* genes into pKE112-MCS at the *Eco*RI/*Kpn*I and *Kpn*I/*Bam*HI sites, respectively. The *P. putida* ATCC12633 *davAB* genes were synthesized at Bioneer based on the reported sequence. pKE112-DavABGabTD was constructed by sequentially cloning the *P. putida* KT2440 *gabT* and *gabD* genes into pKE112-DavAB at the *Bam*HI/*Sbf*I and *Sbf*I/*Hin*dIII sites, respectively (Fig. 2).

2.3. Culture conditions and 5AVA and glutarate analyses

E. coli XL1-Blue was cultured at 37 °C in Luria–Bertani (LB) medium containing (per liter): 10 g tryptone, 5 g yeast extract,

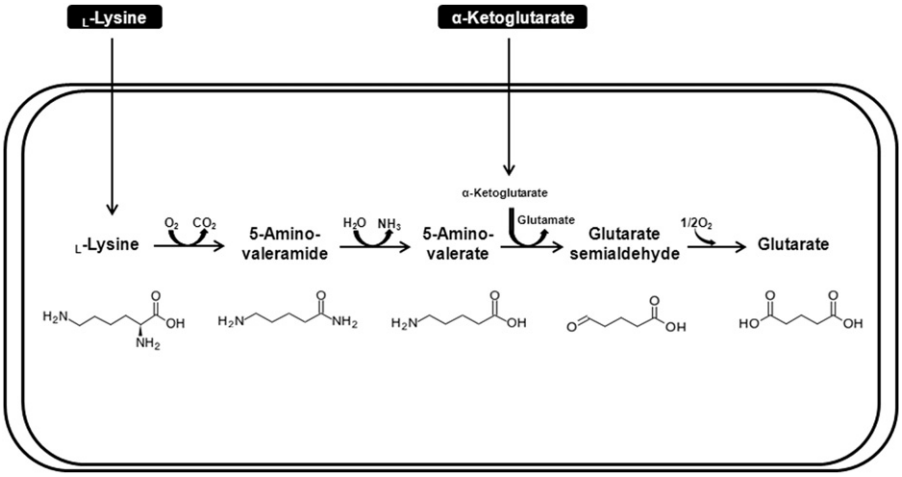

Fig. 1. Metabolic pathways for the production of 5AVA and glutarate from l-lysine. For the validation of the pathway, 10 g/L of L-lysine was added to the culture medium as the precursor for 5AVA when the wild-type *E. coli* strain was used as a host strain. For the production of glutarate, 10 g/L of α-ketoglutarate was added to the culture medium along with 10 g/L of L-lysine to facilitate transamination of 5-AVA.

Table 1
Lists of strains and plasmids used in this study.

Plasmid	Relevant characteristics	Reference or source
Strains		
XL1-Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F′ *proAB lacI*^{q}*ZΔM15* Tn*10* (Tet^R)]	Stratagene[a]
WL3110	W3110 *ΔlacI*	Park et al. (2007)
XQ56	W3110 *ΔlacI ΔspeE ΔspeG ΔygjG ΔpuuPAPdapA*::P*trc*	Qian et al. (2011)
Plasmids		
pKE12-MCS	Expression vector; $P_{LlacO-1}$ promoter, *Ralstonia eutropha* PHA biosynthesis genes transcription terminator; Ap^r	Park et al. (2012b)
pTacLacI	Expression vector; *tac* promoter; Ap^r	Lee et al. (2008)
pKE112-MCS	Expression vector; *tac* promoter, *Ralstonia eutropha* PHA biosynthesis genes transcription terminator; Ap^r	This study
pKE112-DavAB	pKE112-MCS derivative; *P. putida davAB*; Ap^r	This study
pKE112-DavABGabTD	pKE112-MCS derivative; *P. putida davAB, P. putida gabTD*; Ap^r	This study

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