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Modification of aspartokinase III and dihydrodipicolinate synthetase increases the production of L-lysine in *Escherichia coli*

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ABSTRACT

To develop an L-lysine high-yielding strain, the enzymes involved in the L-lysine biosynthetic pathway, including aspartokinase III (AK III) and dihydrodipicolinate synthetase (DHDPS) were investigated. Allosteric enzymes involved in L-lysine production from L-lysine producer *Escherichia coli* LATR11 were sequenced and showed that AK III with mutation T344M or DHDPS with mutation H56K is more conducive to L-lysine production than AK III with mutation M318I or E250K-M318I-G323D or DHDPS with mutation H118Y. Moreover, an L-lysine high-yielding strain was developed from *E. coli* LATR11 via overexpression of *ppc*, $lysC^{T344M}$, *asd*, $dapA^{H56K}$, *dapB*, and *lysA* combined with heterologous expression of *Corynebacterium glutamicum ddh*. The resulting strain LATR11/pWG-$DC^{SM}A^{SM}BH_{c.g}LP$ showed high L-lysine production ($37.2 \pm 2.3\ g\ L^{-1}$) with productivity (Q_P) of $1.16\ g\ L^{-1}\ h^{-1}$ in shake flasks. In fed-batch fermentation, LATR11/pWG-$DC^{SM}A^{SM}BH_{c.g}LP$ produced about $125.6\ g\ L^{-1}$ of L-lysine with Q_P of $3.14\ g\ L^{-1}\ h^{-1}$ and glucose conversion rate (α) of 58.97% after 40 h. From which we got the following conclusions: the enzyme with non-feedback control and high activity, and the high flux through biosynthetic pathway are beneficial to improve L-lysine production in *E. coli*. These results provide a definite theoretical foundation for breeding amino acid high-yielding strains via genetic engineering from classical producers.

1. Introduction

L-Lysine, one of the essential amino acids required for nutrition in animals and humans [1], is widely used in the feed industry, medical industry, *etc*. L-lysine has been mainly produced by microbial fermentation employing mutant strains of bacteria, such as *Corynebacterium* sp. and *Escherichia* sp. [2]. With L-aspartate as the precursor, the biosynthetic pathway of L-lysine is composed of two pathways: aspartate pathway and diaminopimelate (DAP) pathway in *E. coli* (Fig. 1; [3]). The aspartate pathway contains two steps that are catalyzed by aspartokinase (AK) and aspartate semialdehyde dehydrogenase (ASADH), respectively [3]. Moreover, the aspartate pathway is shared for the biosynthesis of many amino acids that are required for protein biosynthesis in organisms [4]. The DAP pathway consists of six steps, and dihydrodipicolinate synthase (DHDPS) catalyzes this first step in the DAP pathway (Fig. 1). As the first enzyme in the pathway, AK and DHDPS catalyze the rate-limiting step in L-lysine biosynthesis. Therefore, AK and DHDPS are potential targets for metabolic engineering.

In *E. coli*, there are three types of AK isozymes (*i.e.*, AK I, AK II, and AK III), which catalyze the conversion of L-aspartate to L-aspartyl-phosphate and are the key regulatory enzymes for aspartate-type amino acids biosynthesis [5]. AK I (encoded by *thrA*) and AK II (encoded by *MetL*) are bifunctional enzymes, whereas AK III (encoded by *lysC*) is a monofunctional enzyme [6]. AK III has a homodimeric structure and the N-terminal domain is responsible for catalytic activity of the holoenzyme, while the C-terminal domain contains the regulatory domain [6]. It is well known that the transcription of *lysC* and the activity of AK III are regulated by L-lysine [3]. Fortunately, the mutant strains with lysine-insensitive AK III have been isolated by selecting for resis-

Abbreviations: DAP, diaminopimelate; AEC, *S*-(2-aminoethyl)-L-cysteine; AEC^{hr}, AEC high resistant; Km, kanamycin; Rif, rifampicin; DCW, dry cell weight; LB, Luria-Bertani; LBG, LB + 5 g L^{-1} glucose; LBS, LB + 100 g L^{-1} sucrose; LBK, LB + 50 μg mL^{-1} Km; LBHIS, LB + brain heart infusion + sorbitol; α, conversion rate of glucose.

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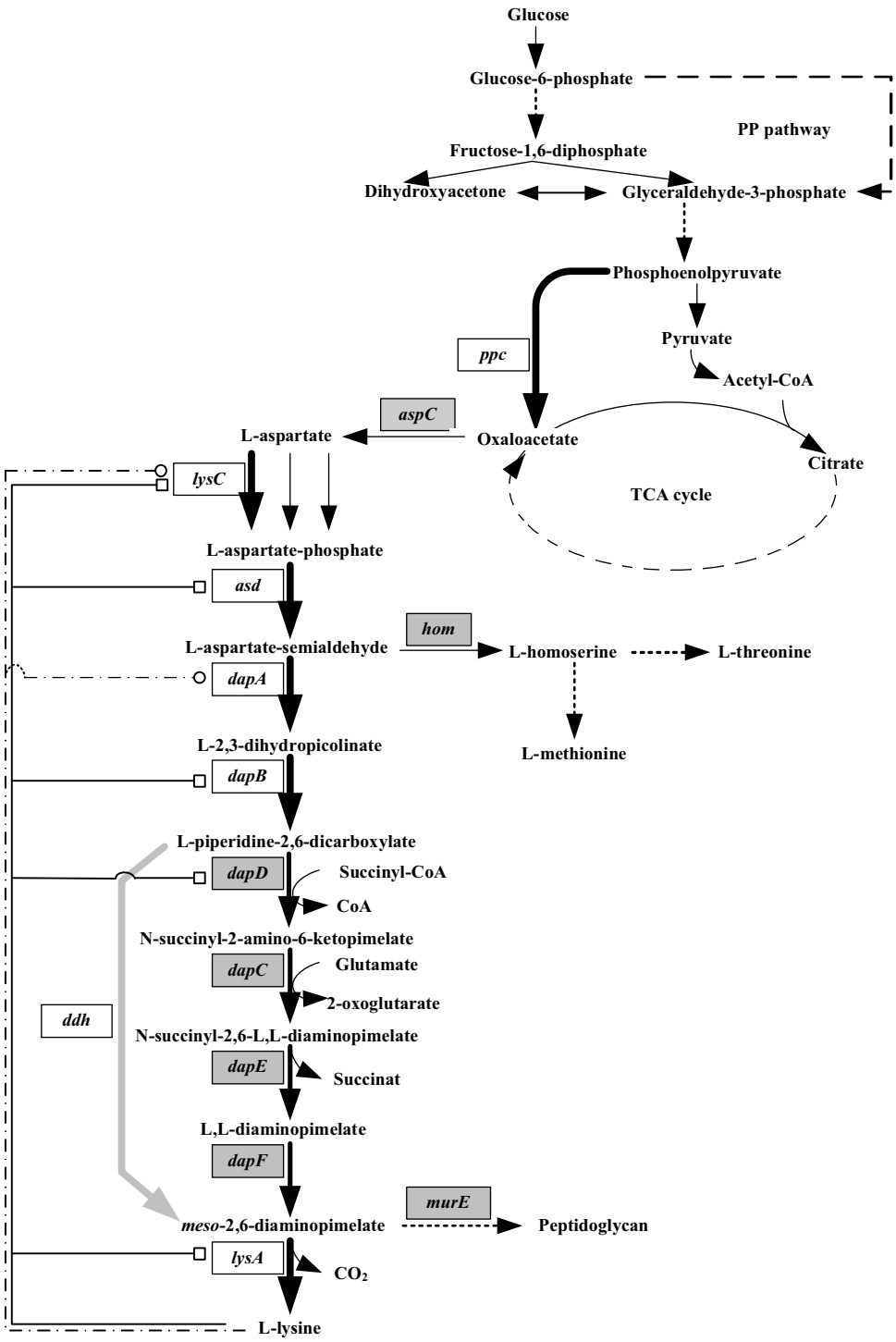

Fig. 1. Schematic of metabolic engineering strategy for genetically modifying *E. coli* strain for L-lysine production. The thick arrows and genes in white graph box represent up-regulated pathway and overexpressed genes, respectively. The gray line and dotted lines represents extrinsic reaction and omitted steps, respectively. represents inhibition reaction and represents repress reaction.

tance to *S*-(2-aminoethyl)-L-cysteine (AEC) after multiple rounds of random mutagenesis, because AK III from mutant strains has missense mutations in amino acid residues 250 or 323–352 [5]. Because mutagenesis is a random event, adverse base substitution is inevitable in mutant strain with the desired properties. For example, enzyme activity is decreased compared with the wild-type enzyme [5,7]. In summary, lysine-insensitive and high-activity AK III is most important for L-lysine fermentation in *E. coli*.

DHDPS (encoded by *dapA*), another rate-limiting enzyme in *E. coli*, catalyzes the condensation of pyruvate and L-aspartate semialdehyde (ASA) to form L-2,3 dihydrodipicolinate (DHDP) (Fig. 1; [8]). The native *E. coli* DHDPS is a homotetramer and is composed of a dimer of "tight dimers" [9,10]. Each monomer of DHDPS consists of two domains: N-terminal domain is a parallel $(\beta/\alpha)8$-barrel with the active site and L-lysine binding site, whereas C-terminal domain is composed of three α-helices [11]. C-terminal domain plays an important role in maintenance of enzyme activity and

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