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Co-production of *S*-adenosyl-L-methionine and L-isoleucine in *Corynebacterium glutamicum*

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ABSTRACT

In this study, production of *S*-adenosyl-L-methionine in *Corynebacterium glutamicum* was investigated by overexpressing genes *metK* and *vgb*. Compared with vector control, overexpression of *metK* alone in *C. glutamicum* ATCC13032 and IWJ001 increased SAM production 5.11 and 11.65 times, respectively; while overexpression of *metK* and *vgb* in *C. glutamicum* ATCC13032 and IWJ001 increased SAM production 5.83 and 14.95 times, respectively. Further studies on IWJ001/pDXW-8-*metk-vgb* showed that the limiting factor for SAM production is intracellular ATP supply. Since IWJ001 is an L-isoleucine production strain, IWJ001/pDXW-8-*metk-vgb* could produce both SAM and L-isoleucine. After 72 h fermentation, SAM and L-isoleucine in IWJ001/pDXW-8-*metk-vgb* reached 0.67 g/L and 13.8 g/L, respectively. The results demonstrate the potential application of *C. glutamicum* for co-production of SAM and amino acids.

1. Introduction

S-Adenosyl-L-methionine (SAM), a methyl group donor, is important in all living organisms. SAM has been used for prescription medication dietary supplement and treatment of depression, liver hepatitis, schizophrenia osteoarthritis [1–3]. Because of the increasing demand of SAM, it is urgent to develop efficient ways for SAM production.

SAM can be synthesized from methionine and ATP by methionine adenosyltransferase (MAT) in many microorganisms. Microbial fermentation is ideal for SAM production [4]. *Pichia pastoris* [4] and *Saccharomyces cerevisiae* [5] overexpressing *SAM2* could produce 2.49 and 2.8 g/L SAM, respectively. The recombinant *P. pastoris* could produce 13.5 g/L after 100 h fermentation in 5 L fermenter [6]. *S. cerevisiae* or *P. pastoris* could efficiently produce SAM because MAT activity encoded by SAM2 in these microorganisms is not inhibited by SAM, but the expensive L-methionine has to be added, thus raising the production cost. Overexpression of *vgb*, the gene encoding *Vitreoscilla* hemoglobin (VHb) could also improve SAM yield in *P. pastoris* [7], possibly because VHb had effects on the reduced oxygen availability, promoted cell growth and protein expressions [8–10], improved the electron transport chain, enhanced ATP synthesis rate and aerobic respiration efficiency [11–13]. In the H^+-ATPase-defective mutant of *Corynebacterium glutamicum* ATCC 14067, the expression levels of *pyk* encoding pyruvate kinase, *mqo* encoding malate:quinine oxidoreductase, and *mdh* encoding malate dehydrogenase increased, contributing to the reoxidation of NADH during the increased respiration and enhanced glucose metabolism in response to cellular energy shortage [14]; 3-phosphoglycerate kinase encoded by *pgk* in glycolysis is also important for enhancing ATP substrate level phosphorylation [14]. Therefore, overexpression of *mdh*, *mqo*, *pyk*, and *pgk* might provide more energy for SAM production in *C. glutamicum*.

C. glutamicum is well known for its ability to produce various amino acids [15–19] and other products such as γ-aminobutyrate [20], cadaverine [21], ethanol [22], and organic acids [23]. Overexpressing *metK* encoding MAT in *C. glutamicum* ATCC 13032 could increase the intracellular SAM concentration [24]. In *C. glutamicum*, L-homoserine is the common key precursor for both SAM and L-isoleucine production (Fig. 1). Since SAM is an intracellular product and amino acids are extracellular products, *C. glutamicum* could be ideal for co-production of SAM and amino acids, and thus reducing the production cost.

In this study, *metK* was cloned into pDXW-8, the shuttle vector between *Escherichia coli* and *C. glutamicum*, and overexpressed in *C. glutamicum* ATCC 13032 and IWJ001, an L-isoleucine production strain. The gene *metK* can be overexpressed in both *C. glutamicum*

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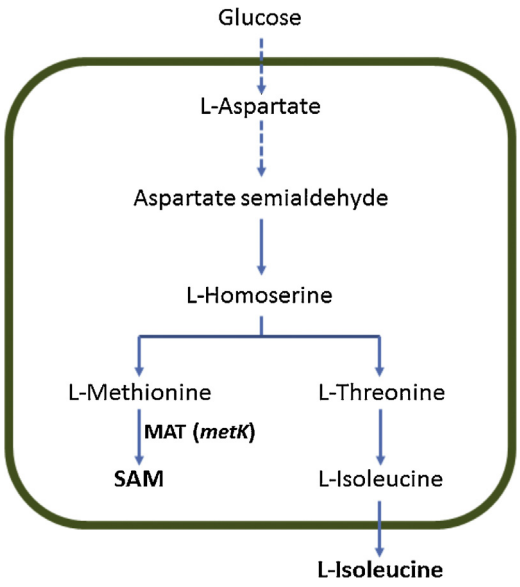

Fig.1. Biosynthetic pathways of SAM and L-isoleucine in *C. glutamicum*.

strains, but more SAM was produced in *C. glutamicum* IWJ001. Since *C. glutamicum* IJW001 is an L-isoleucine production strain, IWJ001/pDXW-8-*metk* produced both SAM and L-isoleucine. Co-overexpression of *metK* and *vgb* encoding VHb further improved SAM yield in *C. glutamicum* IWJ001. In flask cultivation, SAM and L-isoleucine reached 0.65 g/L and 4.75 g/L, respectively; in fed-batch cultivation, SAM and L-isoleucine reached 0.67 g/L and 13.8 g/L, respectively. Although the SAM yield in *C. glutamicum* IWJ001 is not as high as in *P. pastoris*, this is the highest yield in *C. glutamicum*, and L-isoleucine was also produced. The results demonstrated the feasibility for co-production of SAM and L-isoleucine in *C. glutamicum.*

2. Materials and methods

2.1. Construction of strains and plasmids

The expression stains and plasmids used in this study are summarized in Table 1. *E. coli* strain DH5α was used for plasmid construction. The gene *metK* was amplified from *C. glutamicum* ATCC13032. The primers used in this study (Table 2) were designed according to the genome sequence of *C. glutamicum* ATCC 13032 [25]. The gene *metK* was PCR amplified by using primer pair *metK*-F and *metK*-R; the PCR product was digested with *EcoR* I and *Sac* I, and ligated into the shuttle vector pDXW-8 [26], resulting in pDXW-8-*metK*. The *vgb* gene was amplified from plasmid pDXW-8-*vgb* [26] with the primers *vgb*-F and *vgb*-R; the *vgb* fragment was then cloned into pDXW-8-*metK*, forming pDXW-8-*metK-vgb*. Plasmids were transformed into *C. glutamicum* IWJ001 or ATCC13032, resulting strains IWJ001/pDXW-8, IWJ001/pDXW-8-*metk*, IWJ001/pDXW-8-*metk-vgb*, and ATCC13032/pDXW-8, ATCC13032/pDXW-8-*metk,* ATCC13032/pDXW-8-*metk-vgb*.

The genes *mdh*, *mqo*, *pyk*, and *pgk* were cloned from the genome of *C. glutamicum* ATCC13032. Primer pair *mdh*-F and *mdh*-R were used to amplify *mdh*; primer pair *mqo*-F and *mqo*-R were used to amplify *mqo*; primer pair *pyk*-F and *pyk*-R were used to amplify *pyk*; and primer pair *pgk*-F and *pgk*-R were used to amplify *pgk*. PCR product of *mdh* was digested with *Xho* I and *Bgl* II, PCR product of *mqo* was digested with *Bgl* II and *Afl* II, PCR product of *pyk* was digested with *Sac* II and *Afl* II, PCR product of gene *pgk* was digested with *Bgl* II and *Hind* III; then ligated into pDXW-8-*metK-vgb* that was similarly digested, resulting in plasmids pDXW-8-*metK-vgb-mdh*, pDXW-8-*metK-vgb-mqo*, pDXW-8-*metK-vgb-pyk*; and pDXW-8-*metK-vgb-pgk*, respectively. These plasmids were transformed into *C. glutamicum* IWJ001, resulting in strains IWJ001/pDXW-8-*metK-vgb-mdh*, IWJ001/pDXW-8-*metK-vgb-mqo*, IWJ001/pDXW-8-*metK-vgb-pyk*, and IWJ001/pDXW-8-*metK-vgb-pgk*.

E. coli cells were grown in LB medium (5 g/L yeast extract, 10 g/L tryptone and 10 g/L NaCl) at 37 °C and 200 rpm, and the competent cells were prepared according to the published method [27].

C. glutamicum IWJ001, an L-isoleucine producer obtained by multiple random mutagenesis, has been deposited in China center for type culture collection with the strain number CCTCC M2014493. *C. glutamicum* IWJ001 and ATCC13032 was grown in LBG medium (LB medium supplemented with 5 g/L glucose) at 30 °C and 200 rpm. Modified Epo media (5 g/L yeast extract, 10 g/L tryptone, 10 g/L NaCl, 30 g/L glycine and 0.1% Tween 80) was used for preparing competent cells of *C. glutamicum*. LBHIS media (2.5 g/L yeast extract, 5 g/L tryptone, 5 g/L NaCl, 18.5 g/L brain heart infusion powder and 91 g/L sorbitol) was used for *C. glutamicum* transformation [28]. When necessary, 30 μg/L kanamycin was added in the media for maintaining the plasmid.

Table 1
Bacterial strains and plasmids used in this study.

Strains or plasmids	Description	Sources
Strains		
DH5α	Wild type *E. coli*	Novagen
IWJ001	L-isoleucine producing strain of *C. glutamicum*	CCTCC
ATCC13032	Wild type *C. glutamicum*	ATCC
IWJ001/pDXW-8	IWJ001 harboring pDXW-8	This work
IWJ001/pDXW-8-*metk*	IWJ001 harboring pDXW-8-*metk*	This work
ATCC13032/ pDXW-8-*metk*	ATCC 13032 harboring pDXW-8-*metk*	This work
IWJ001/pDXW-8-*metk-vgb*	IWJ001 harboring pDXW-8-*metk-vgb*	This work
IWJ001/pDXW-8-*metk-vgb-mdh*	IWJ001 harboring pDXW-8-*metk-vgb-mdh*	This work
IWJ001/pDXW-8-*metk-vgb-mqo*	IWJ001 harboring pDXW-8-*metk-vgb-mqo*	This work
IWJ001/pDXW-8-*metk-vgb-pyk*	IWJ001 harboring pDXW-8-*metk-vgb-pyk*	This work
IWJ001/pDXW-8-*metk-vgb-pgk*	IWJ001 harboring pDXW-8-*metk-vgb-pgk*	This work
Plasmids		
pDXW-8	Shuttle vector between *E. coli* and *C. glutamicum*	[26]
pDXW-8-*metk*	pDXW-8 harboring *metK*	This work
pDXW-8-*metk-vgb*	pDXW-8 harboring *metk-vgb*	This work
pDXW-8-*metk-vgb-mdh*	pDXW-8 harboring *metk-vgb-mdh*	This work
pDXW-8-*metk-vgb-mqo*	pDXW-8 harboring *metk-vgb-mqo*	This work
pDXW-8-*metk-vgb-pyk*	pDXW-8 harboring *metk-vgb-pyk*	This work
pDXW-8-*metk-vgb-pgk*	pDXW-8 harboring *metk-vgb-pgk*	This work