Metabolic engineering for the enhanced biosynthesis of γ-aminobutyric acid (GABA) and α-ketoglutarate in *Corynebacterium glutamicum*

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Abstract

γ-aminobutyric acid (GABA) is widely known as one of the neurotransmitters in the central nerve system. Because of its biological and physiological functions, bio-industries have tried to develop functional foods and pharmaceutical drugs containing GABA, recently. In addition, GABA can be used as a monomer of biopolymers such as bio-plastic. In this study, development of strains for the hyper-production of GABA using glutamate overproducer, *Corynebacterium glutamicum* HH09, was carried out. First of all, *gaba-T* gene encoding gaba-aminotransferase was knocked-out by Cre/*loxP* system to block the bioconversion from GABA to succinate semialdehyde. To the resulting strain (HH104), *gad* gene encoding glutamate decarboxylase (GAD) was introduced for the accumulation of GABA in *C. glutamicum* (HH105). On the other hand, 1 copy of *gad* was integrated into the chromosome of HH104 to make HH106 followed by transformation of plasmid containing *gad* to make 107. The resulting metabolically engineered *C. glutamicum* HH105, 106 and HH107 produced 162 g/L, 140 g/L and 199 g/L by 2.5 L fed-batch fermentations, respectively.

The development of microbial strains for the enhanced production of α-ketoglutarate (α-KG) was investigated using a strain of *Corynebacterium glutamicum* that overproduces of L-glutamate, by disrupting three genes involved in the α-KG biosynthetic pathway. The pathways competing with the biosynthesis of α-KG were blocked by knocking out *aceA* (encoding isocitrate lyase, ICL), *gdh* (encoding glutamate dehydrogenase, L-gluDH), and *gltB* (encoding glutamate synthase or glutamate-2-oxoglutarate aminotransferase, GOGAT). The strain with *aceA*, *gltB*, and *gdh* disrupted showed reduced ICL activity and no GOGAT and L-gluDH activities, resulting in up to 16-fold more α-KG production than the control strain in flask culture. These results suggest that L-gluDH is the key enzyme in the conversion of α-KG to L-glutamate; therefore, prevention of this step could promote α-KG accumulation. The inactivation of ICL leads the carbon flow to α-KG by blocking the glyoxylate pathway. However, the disruption of *gltB* did not affect the biosynthesis of α-KG. In the fed-batch cultures using 5 L-jar fermentor, the strain JH107 (*gdh gltB* double mutant) and JH110 (*aceA gltB gdh* triple mutant) produced 51.1 g/L and 47.9 g/L of α-KG, respectively. The production of α-KG will be improved if further fermentation conditions focused on the strain JH110. Our results can be applied in the industrial production of α-KG by using *C. glutamicum* as producer.

Biography

Hyune-Hwan Lee has completed his Ph.D at Department of Zoology, College of Natural Sciences, Seoul National University, Seoul, Korea in 1990. He held the CEO position at Digital Biotech Inc. (Bio-Venture company) from year 1999 to year 2002. Hyune-Hwan Lee was a visiting research professor at Institute of Biotechnogy-1 (IBI-1), Forschungzentrum, Juelich, Germany (2001) and at the Julia McFarlane Diabetes Institute of Medical School, University of Calgary (2002 – 2003).