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Metabolic engineering of *Escherichia coli* for 1-butanol and 1-propanol production via the keto-acid pathways

C.R. Shen, J.C. Liao *

Department of Chemical and Biomolecular Engineering, University of California, 5531 Boelter Hall, Los Angeles, CA 90095, USA

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ABSTRACT

Production of higher alcohols via the keto-acid intermediates found in microorganism's native amino-acid pathways has recently shown promising results. In this work, an *Escherichia coli* strain that produces 1-butanol and 1-propanol from glucose was constructed. The strain first converts glucose to 2-ketobutyrate, a common keto-acid intermediate for isoleucine biosynthesis. Then, 2-ketobutyrate is converted to 1-propanol through reactions catalyzed by the heterologous decarboxylase and dehydrogenase, or to 1-butanol via the chemistry involved in the synthesis of the unnatural amino acid norvaline. We systematically improved the synthesis of 1-propanol and 1-butanol through deregulation of amino-acid biosynthesis and elimination of competing pathways. The final strain demonstrated a production titer of 2 g/L with nearly 1:1 ratio of butanol and propanol.

1. Introduction

The shortage of petroleum and the environmental perturbation due to its consumption have become a crucial problem facing the world in this century. In an attempt to lower the petroleum demand and to utilize renewable resources, bio-ethanol production has been intensively studied over the past 50 years. Despite the current use of ethanol as a transportation fuel, interest in butanol as the next-generation gasoline substitute has grown because of its higher energy density and lower vapor pressure compared to ethanol. In addition, its lower hygroscopicity allows it to be readily stored and distributed using existing infrastructure.

1-Butanol production from carbohydrates has been carried out using *Clostridium* through acetone–butanol–ethanol (ABE) fermentation (Lin and Blaschek, 1983; Nair and Papoutsakis, 1994; Formanek et al., 1997). However, *Clostridium*'s complex physiology and difficulty for genetic manipulation present challenges for further improvement in this organism. It is thus of interest to transfer the butanol production pathway from *Clostridium* to an easily manipulated organism, such as *Escherichia coli*. The initial success of this task has recently been demonstrated (Atsumi et al., 2007).

1-Propanol is another alcohol that can potentially be used as a gasoline substitute. It is currently used as a multi-purpose solvent in a variety of industrial products such as paint, cleaner and cosmetics. Microbial production of 1-propanol has been detected from certain species of *Clostridium* (Janssen, 2004) via threonine catabolism and from yeast (Eden et al., 2001) in beer fermentation. However, both resulted in only small quantities of 1-propanol (<70 mg/L). No existing microorganism has been reported to produce 1-propanol from sugars in significant amounts.

Instead of using the pathways naturally evolved for alcohol production in microorganisms, our group has devised a systematic approach (Atsumi et al., 2008) for the synthesis of higher alcohols utilizing the amino-acid biosynthetic pathways that are present in all organisms. Not only is this system readily transferable into other hosts but utilization of native amino-acid intermediates as alcohol production precursors also minimizes metabolic perturbation caused by toxic intermediates. With this strategy, Atsumi et al. (2008) have demonstrated a high level of isobutanol production in *E. coli*. Here, the same strategy is applied to produce 1-butanol and 1-propanol in *E. coli*.

As reported earlier (Atsumi et al., 2008), upon introduction of the promiscuous 2-ketoacid decarboxylase (Kivd) from *Lactococcus lactis* (Smit et al., 2005) and alcohol dehydrogenase 2 (ADH2) from *Saccharomyces cerevisiae* into *E. coli*, 2-ketobutyrate can be converted into 1-propanol (Fig. 1a). While 2-ketobutyrate is a common intermediate derived from threonine and a precursor for isoleucine biosynthesis, the 1-butanol precursor 2-ketovalerate is a rare metabolite in the cell leading to the synthesis of the unnatural amino acid, norvaline. Similar to the formation of 2-ketoisocaproate (McCourt and Duggleby, 2006), the precursor for leucine biosynthesis, production of 2-ketovalerate was catalyzed by the enzymes LeuABCD using 2-ketobutyrate as an alternative starting substrate (Fig. 1b) via the keto-acid chain elongation process (Bogosian et al., 1989; Kisumi et al., 1976).

* Corresponding author. Fax: +1 310 206 4107.
E-mail address: liaoj@seas.ucla.edu (J.C. Liao).

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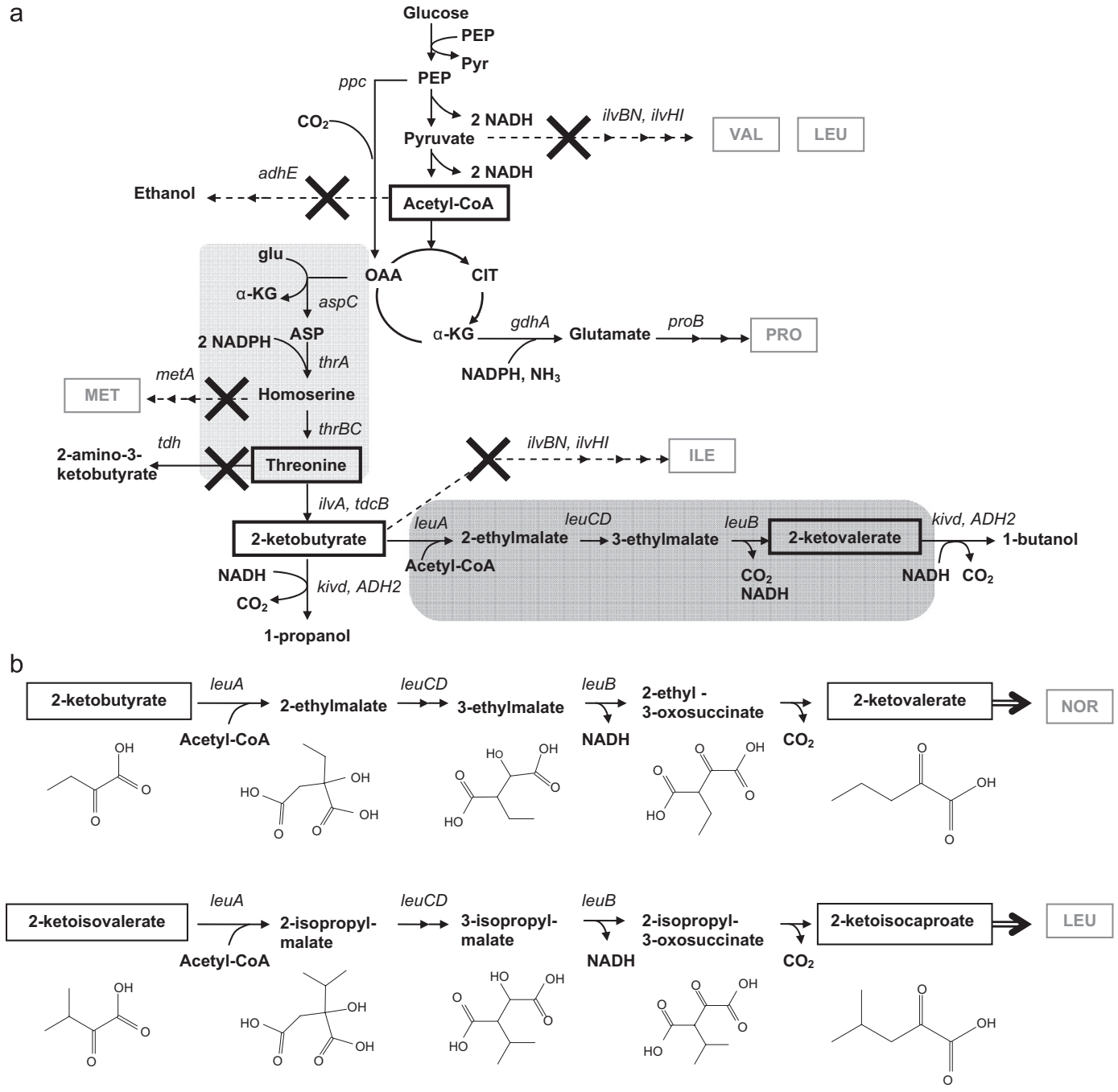

Fig. 1. (a) Schematic illustration of 1-propanol and 1-butanol production via the threonine and norvaline biosynthetic pathways in the genetically engineered *E. coli*. Disruptions of the specific pathways are indicated by the crosses. Rectangular boxes with thick lines are placed around the essential precursors for the alcohol production. The threonine pathway is shaded with light gray while the norvaline pathway is shaded with dark gray. Pathways leading to the biosynthesis of valine, leucine, isoleucine, proline and methionine are indicated by their corresponding abbreviations. (b) Reactions of 2-ketoacids catalyzed by the leucine biosynthetic enzymes LeuABCD. Top panel shows the synthesis of 1-butanol precursor 2-ketovalerate utilizing the unnatural norvaline pathway. Bottom panel shows the synthesis of leucine precursor 2-ketoisocaproate. 2-ketoisovalerate is the natural substrate for LeuA. The 2-ketoacids are enclosed by rectangular boxes.

In the proposed pathway, 2-isopropylmalate synthase (LeuA) is responsible for the Aldol addition of acetyl CoA to 2-ketobutyrate, which differs from its natural substrate 2-ketoisovalerate by a methyl group at the beta position (Fig. 1b). Then, Isopropylmalate isomerase, consisting of two subunits LeuC and LeuD, catalyzes the transfer of the hydroxyl group between adjacent carbons, converting 2-ethylmalate into 3-ethylmalate. Finally, oxidation and decarboxylation of 3-ethylmalate are performed by the metal-dependent 3-isopropylmalate dehydrogenase (LeuB) using NAD+ as the electron acceptor to yield 2-ketovalerate, NADH and CO_2. Instead of being transaminated into norvaline, the resulting 2-ketovalerate is subsequently turned into 1-butanol by Kivd and ADH2 (Fig. 1a).

In this work, we achieved co-production of 1-butanol and 1-propanol by metabolically engineering *E. coli*. This work demonstrates that the vast amount of knowledge accumulated for amino-acid hyper-productions can be readily transferred and applied to the production of higher alcohols such as 1-propanol