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Recent advances in microbial production of fuels and chemicals using tools and strategies of systems metabolic engineering

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ABSTRACT

The advent of various systems metabolic engineering tools and strategies has enabled more sophisticated engineering of microorganisms for the production of industrially useful fuels and chemicals. Advances in systems metabolic engineering have been made in overproducing natural chemicals and producing novel non-natural chemicals. In this paper, we review the tools and strategies of systems metabolic engineering employed for the development of microorganisms for the production of various industrially useful chemicals belonging to fuels, building block chemicals, and specialty chemicals, in particular focusing on those reported in the last three years. It was aimed at providing the current landscape of systems metabolic engineering and suggesting directions to address future challenges towards successfully establishing processes for the bio-based production of fuels and chemicals from renewable resources.

Contents

1. Introduction

Growing concerns on climate changes caused by petroleum-based industries have attracted much attention in establishing biosustainability from various industries around the globe. Biorefineries aim at replacing as many unit processes of the petroleum-based refineries as possible, particularly through employing engineered microorganisms; that is to metabolically engineer microbial hosts to overproduce industrially useful chemicals and fuels from renewable biomass, thus moving away from fossil-resource dependent way of living.

Systems metabolic engineering, which integrates metabolic engineering with systems biology, synthetic biology and evolutionary engineering in the context of the entire bioprocess, has contributed to developing efficient strategies to improve microbial strains overproducing fuels and chemicals (Becker et al., 2011; Lee et al., 2011b, 2012). Systems metabolic engineering attempts to develop microbial strains on the basis of whole optimized bioprocess in order to maximize the production yield and productivity of the target chemical, while minimizing overall operation costs

Abbreviations: 1,4-BDO, 1,4-butanediol; 2,3-BDO, 2,3-butanediol; 3HP, 3-hydroxypropionate; 5AVA, 5-aminovaleric acid; ABE, acetone-butanol-ethanol; CASOP, computational approach for strain optimization aiming at high productivity; FAEE, fatty acid ethyl esters; FASEs, fatty acid short-chain esters; GlcNAc, N-acetylglucosamine; SCAs, short-chain alkanes; SDO, styrene dioxygenase; SMO, styrene monooxygenase; sRNAs, synthetic small RNAs.

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that incur throughout the upstream and downstream processes. For this grand challenge, intracellular metabolic fluxes are optimized towards the overproduction of the target chemical by using various molecular and high-throughput techniques, including, but not limited to: conventional gene knockout and overexpression (Jang et al., 2012), construction of a novel metabolic pathway using promiscuous enzymes (Atsumi et al., 2008; Shen et al., 2011), sophisticated downregulation of gene expression levels (Na et al., 2013; Yoo et al., 2013), multiple enzyme targets (Flowers et al., 2013), multiple genome engineering (Isaacs et al., 2011; Wang et al., 2009), synthetic regulatory circuits (Thieffry, 2007), omics analysis (Park et al., 2007), and *in silico* modeling and simulation (Yim et al., 2011). Because cultivation conditions are equally critical to the microbial strain's production performance, they are also thoroughly considered, including medium composition (Song et al., 2008; Thompson and Trinh, 2014), cultivation modes (i.e., batch versus fed-batch) (Park et al., 2011b), pH (Zhu et al., 2007), and aeration (Causey et al., 2003). Optimization of these bioprocess variables can significantly contribute to minimizing the overall operation costs.

Based on recent successful examples of developing microorganisms using the tools and strategies of systems metabolic engineering, we herein review recent trends in microbial production of various chemicals, including fuels, building blocks (or monomers), and specialty chemicals (Fig. 1; Table 1). Although chemicals discussed in this paper are classified into one of these three chemical types, they can also be assigned to different types (Curran and Alper, 2012). In this review, a particular focus is given to those systems metabolic engineering studies reported in the last three years.

2. Microbial production of fuels

Microbial production of fuels initially started with ethanol fermentation obviously due to the availability of technologies for large-scale fermentation that have existed for a long time (Ha et al., 2011; Jarboe et al., 2007; Trinh et al., 2008). With the advent of precise molecular tools and increasing information on biological resources (e.g., microbial strains, genes, and software tools), microorganisms have been successfully engineered to produce different types of fuels, often referred to as advanced biofuels. For instance, microbial production of higher alcohols was successfully demonstrated by using heterologously expressed promiscuous enzymes (Atsumi et al., 2008). More recently, systems metabolic engineering has contributed to the production of 1-propanol, 1-butanol, other higher alcohols, hydrocarbons (alkane or alkene), and biodiesel.

1-Propanol is an important chemical used as a gasoline substitute and in various industrial products (Shen and Liao, 2008). Previously, engineered *Escherichia coli* used 2-ketobutyrate as a key metabolic intermediate for the production of 1-propanol (Atsumi and Liao, 2008; Choi et al., 2012; Shen and Liao, 2008, 2013), while recently developed *Propionibacterium freudenreichii* and *Thermobifida fusca* strains utilizes propionyl-CoA as a key intermediate (Ammar et al., 2013; Deng and Fong, 2011). Among these studies, Choi et al. (2012) concentrated carbon fluxes to 2-ketobutyrate through an amino acid biosynthetic pathway by releasing its feedback inhibition and deleting competing metabolic pathways in *E. coli*. A modified *adhE* gene was subsequently introduced, and a stress response gene was deleted, resulting in the

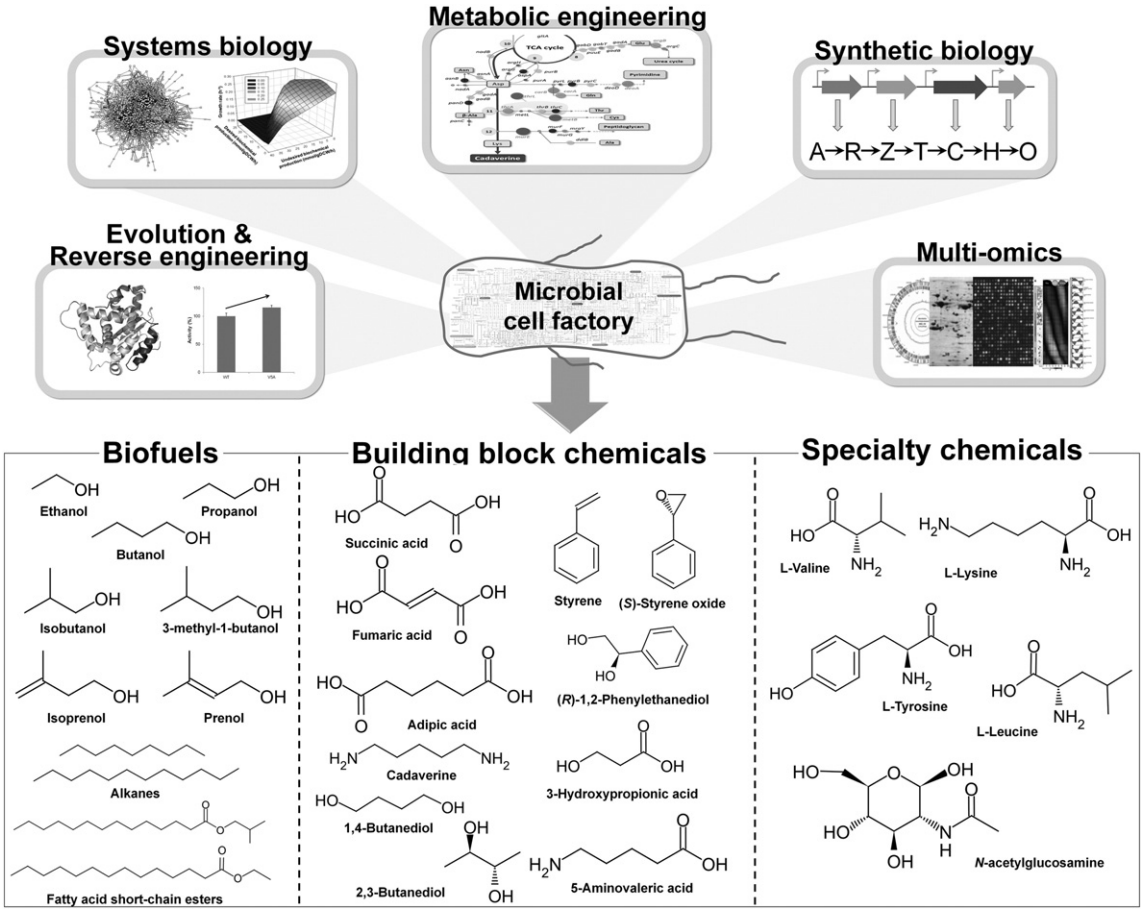

Fig. 1. Representative biofuels, building block chemicals and specialty chemicals using principles of systems metabolic engineering.