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Recent trends in metabolic engineering of microorganisms for the production of advanced biofuels

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As climate change has become one of the major global risks, our heavy dependence on petroleum-derived fuels has received much public attention. To solve such problems, production of sustainable fuels has been intensively studied over the past years. Thanks to recent advances in synthetic biology and metabolic engineering technologies, bio-based platforms for advanced biofuels production have been developed using various microorganisms. The strategies for production of advanced biofuels have converged upon four major metabolic routes: the 2-ketoacid pathway, the fatty acid synthesis (FAS) pathway, the isoprenoid pathway, and the reverse β-oxidation pathway. Additionally, the polyketide synthesis pathway has recently been attracting interest as a promising alternative biofuel production route. In this article, recent trends in advanced biofuels production are reviewed by categorizing them into three types of advanced biofuels: alcohols, biodiesel and jet fuel, and gasoline. Focus is given on the strategies of employing synthetic biology and metabolic engineering for the development of microbial strains producing advanced fuels. Finally, the prospects for future advances needed to achieve much more efficient bio-based production of advanced biofuels are discussed, focusing on designing advanced biofuel production pathways coupled with screening, modifying, and creating novel enzymes.

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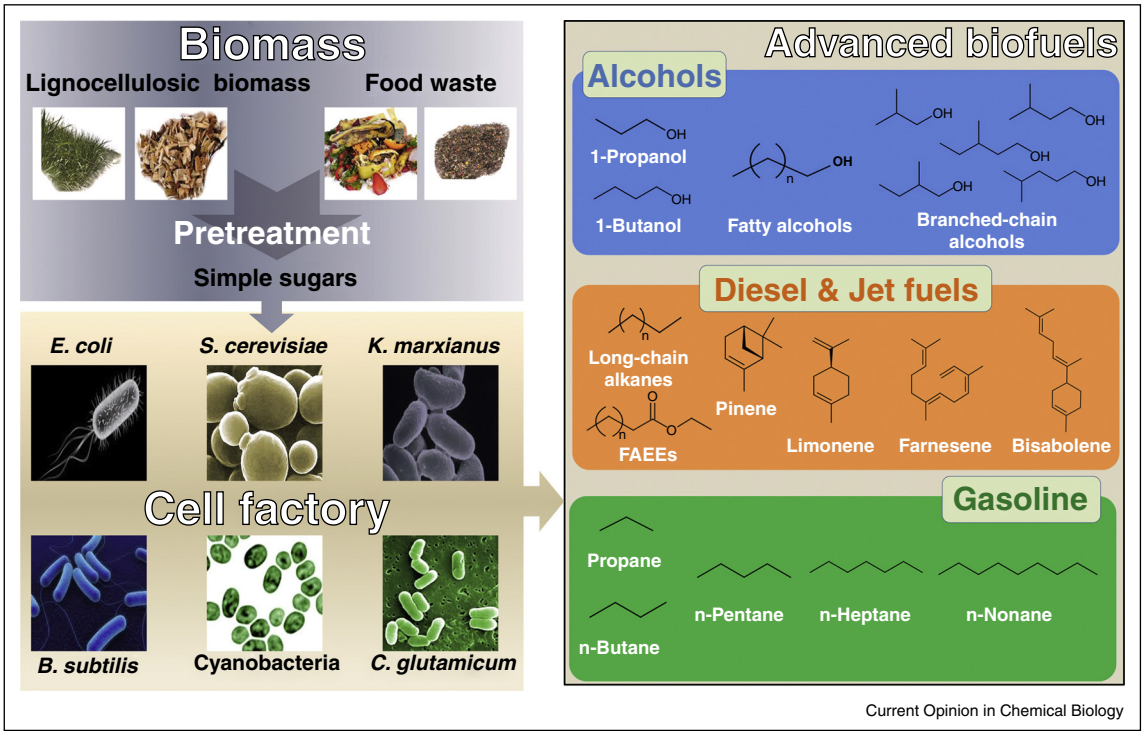
Introduction

Future energy security and the ongoing climate change are urging us to develop more sustainable energy alternatives including biofuels produced from renewable biomass. Today's most representative biofuel is bioethanol fermented from corn or sugarcane [1]. However, bioethanol possesses several undesirable characteristics, including low energy content, hygroscopy, and high vapor pressure [2], leading to the development of advanced biofuels having much better fuel properties (Figure 1). Also, much effort has been exerted to use non-food feedstocks such as oleaginous microalgae, greenhouse gases, or wastes derived from human activities, instead of food crop-derived feedstocks [3,4,5]. Speaking of waste-derived feedstocks particularly, European countries have given attention to agricultural and forest industry wastes for the production of renewable biofuels [6]. Furthermore, a large amount of food waste generated in Asian and Asia-pacific countries has the potential of becoming a versatile feedstock for production of advanced biofuels [7,8•]. Recent advances in metabolic engineering and synthetic biology have accelerated the capability to engineer various microorganisms allowing the engineered microbial strains to efficiently convert such feedstocks into various value-added products, including advanced biofuels.

So far, most metabolic engineering strategies for advanced biofuels production have utilized routes that fall into four major metabolic pathways: the 2-ketoacid pathway (Figure 2a), the fatty acid synthesis (FAS) pathway (Figure 2b), the isoprenoid pathway (Figure 2c), and the reverse β-oxidation pathway (Figure 2b). More recently, the polyketide biosynthetic pathway mediated by polyketide synthases (PKSs) has been tweaked for advanced biofuel production, with results showing good potential (Figure 3).

There have been several review papers on metabolic engineering and synthetic biology strategies employed for designing and optimizing pathways for microbial production of biofuels [1,2,3,4,5]. These recent review articles provide an overview and also a detailed idea for engineering the major metabolic pathways for the production of biofuels including higher alcohols [2] and hydrocarbons [4]. Instead of repeating the information reported in these review papers, we focus on the characteristics and the significance of engineering strategies

Figure 1



Three types of advanced biofuels produced in engineered microorganisms from biomass. Advances in metabolic engineering tools have allowed utilization of a wide array of biomass feedstocks for microbial production of advanced biofuels which are categorized into three types, those are, higher alcohols (blue box), diesel and jet fuel (orange box), and gasoline (green box). Microorganism abbreviations are: *E. coli*, *Escherichia coli*; *S. cerevisiae*, *Saccharomyces cerevisiae*; *B. subtilis*, *Bacillus subtilis*; *K. marxianus*, *Kluyveromyces marxianus*; *C. glutamicum*, *Corynebacterium glutamicum*.

employed for the production of the three major classes of advanced biofuels: higher alcohols, biodiesel and jet fuel, and biogasoline (Figure 1). Higher alcohols contain longer-chain alcohols with or without branched chains, with lower hygroscopy and higher energy content than ethanol, making them suitable as diesel or gasoline substitutes. Biodiesel contains both long-chain fatty acid esters and long-chain alkanes with similar properties as petroleum diesel, while bio-based jet-fuels consist of terpenoid-derived branched-chain or cyclic alkanes. Biogasoline consists of short-chain alkanes identical to those found in petroleum gasoline. Finally, key enzymatic reactions involved in the production pathways are revisited with focuses given on selection and/or evolution of enzymes (Table 1).

Higher alcohols

Despite the drawbacks discussed above, bioethanol has already established a large market size worldwide (Renewable Fuels Association; http://www.ethanolrfa.org/). However, it is expected the bioethanol market will be replaced with more advanced biofuels with better fuel properties such as higher alcohols [2]. Microbial production of primary higher alcohols such as 1-propanol [9,10], 1-butanol [11], and other linear-chain fatty alcohols [2] has been widely studied (Figure 2a and b). Several solventogenic bacterial species belonging to the genus *Clostridium* are well known natural producers of 1-butanol through acetone-butanol-ethanol (ABE) fermentation. Clostridial 1-butanol production, in particular, has a long history, but the process has several limitations as follows. In the ABE fermentation process, co-production of acetone, ethanol and organic acid byproducts reduces the yield and productivity of 1-butanol significantly causing high separation and purification costs [12,13]. More importantly, metabolic engineering of clostridial strains is rather challenging due to the complex metabolic regulation system and difficulties in genetic manipulation. The chronicles and major achievements of clostridial 1-butanol production over the last one hundred years have recently been reviewed [11].

Several studies on 1-butanol production by non-clostridial hosts, including *Escherichia coli* [14,15], *Saccharomyces cerevisiae* [16,17], and *Synechococcus elongatus* [18,19,20], have also been reported. In these studies, the clostridial coenzyme A (CoA)-dependent carbon elongation pathway, also defined in another study as the reverse β-oxidation