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Progress and perspective of biosynthetic platform for higher-order biofuels

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ABSTRACT

The exploitation of innate microbial capacities and/or the importation of novel diverse biosynthetic pathways have become one of the predominant research directions, with both being used to convert fermentable substrates into higher-order biofuels with long carbon chains (> 6) approximating those of gasoline with rating octane value. However, one of the primary issues has been which microorganic biosynthetic platform is most appropriate for transformation into an efficient cell factory for the production of higher-order biofuels. It is indistinct whether such a microorganism would ultimately be engineered using a native, newly isolated strain, a recombinant strain, or a model organism as the starting host. Different biosynthetic platforms microorganisms naturally have different genetic backgrounds, thus presenting different levels of complexity for metabolic networks, the incorporation of different physiological characteristics, cell structural properties, and/or biological activities. These complexities affect strategic formulations of synthetic biology, optimization designs of systems metabolic engineering, selection of metabolic pathways, and operation process difficulties in the realm of evolutionary engineering at the systems level. Here, we offer a global review of existing research for selected, engineered microorganisms designed to produce higher-order biofuels. Our focus on these microorganisms centers on the optimal production of higher-order biofuels using the construction of novel metabolic pathways and/or the alteration of existing pathways as well as examples of their application in recent years. We also discuss potential candidate microorganic biosynthetic platform and offer insight into the circumstances under which each should be used. Finally, we highlight the perspective that developing microorganisms has great possibility, but has not been extensively explored as a viable platform. In this paper, the review is placed in contrast with Crispr-Cas9 genome editing technology that will play an increasingly important role, which can be used to overcome the complex genetic metabolic background of microorganisms at more advanced levels.

1. Introduction

With fossil fuels dwindling supply, increasingly strong association with serious environmental pollution problems and the looming international political issues, as well as developing clean, biosynthetic energy has become a focal point of the scientific community in many countries. In recent years, the continued development of systematic synthetic biology has yielded outstanding achievements in advancing the field of bioenergy through metabolic engineering [1–5]. A diverse set of engineered strains have been developed, which correspond to different target biofuel products from renewable resources [3,6–9]. These have been based on metabolic engineering via importing designed, heterogeneous gene expression pathways or intensively optimized, inherent pathways for each particular microorganism. However, metabolic engineering does not have to rely solely on designed or optimized systems to yield unprecedented metabolic capacity. New, controllable, and feasible metabolic cycle pathways can be achieved via *de novo* synthesis to regulate and balance the metabolic flux in a novel engineered platform microorganism, which can ultimately improve biofuel production.

Abbreviations: KIV, 2-ketoisovalerate; KMV, 2-keto-3-methyl-valerate; TCA, tricarboxylic acid; FAMEs, fatty acid methyl esters; FAEEs, fatty acid ethyl esters; FFAs, Free fatty acids; ACP, acyl carrier protein; AccABCD, ATP-dependent-acetyl-CoA carboxylase; PKS, Polyketides; BGL, β-Glucosidase; CBP, consolidated bioprocessing; CAZy, carbohydrate-active enzymes; 1,3-PD, 1,3-propanediol; OGDC, α-oxoglutarate decarboxylase; SSADH, succinate semialdehyde dehydrogenase; PHB, polyhydroxybutyrate; ADL, L-aspartate β-decarboxylase; DL-ATC, DL-2-amino-Δ2-thiazoline-4-carboxylic acid; TAGs, Triacylglycerols

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With the process of developing bioenergy, increasing carbon length has been a hallmark of the approach. This includes the development of biofuels with short carbon chain from methane to ethanol, butanol, and to higher alcohols, alkane, and alkene compounds that contain more carbon atoms (C > 6). In terms of traditional biomass energy, some native microorganisms can synthesize different kinds of biofuels, including ethanol and butanol via their own metabolic pathways. Ethanol has several drawbacks, including its low energy density, high hygroscopicity, and other factors impeding its use in transportation systems [10,11]. Generally speaking, these factors preclude biofuels with short carbon chain from being an ideal clean energy source to replace gasoline. Since the composition of gasoline is predominantly comprised of C5-C9 alkanes and the octane (C_8H_{18}) value is used as an indicator of anti-explosive performance. When the octane number is higher, the anti-detonating quality is better, thus improving overall engine power. This further adds to reduced fuel consumption, which can lead to less carbon deposits and a higher guarantee of the stability of engine operation. Octane also has a high chemical durability and is not easily decomposed (e.g. redox reaction) during both storage and use, and there is no corrosivity to either the engine parts and/or container. Therefore, if biofuels are created with carbon chain lengths closer to that of octane, the resulting biofuel will have a higher octane number, thus allowing them a greater potential for use as a bioenergy replacement for gasoline. Higher-order biofuels (C > 6) have energy densities and octane numbers comparatively higher and hygroscopicities comparatively lower than other alcohols (C4-C6). They are inherently closer to the nature of gasoline and are the most promising biofuel candidates for replacing gasoline. In addition, some short chain (C5-C6) alkenyl alcohols (e.g. isoprene alcohol) have higher energy when compared with alkane-derived alcohols with the same carbon number. These alkenyl alcohols also have promise as gasoline substitutes. Therefore, one of the major foci for future development of sustainable energy sources is the generation of higher-order biofuels with long carbon chains (C > 6). In recent years, synthetic systems biology has led to the use of alcohols, alkanes and olefins with various carbon chain lengths, for example, C4-C10 higher-order alcohols including isobutanol, 3-methyl-butanol, 2-methyl-butanol, isopentanol, enolates, and C6-C18 alkane or C6-C10 olefins, which were successfully synthesized by non-synthetic microorganisms [12–20].

While the overwhelming majority alcohols (C > 5), such as isobutanol, cannot be synthesized by the native microbe itself, and they could possibly be manufactured by reconstructing metabolic pathways. With this in mind, future research into synthetic biofuels will focus heavily on the development of higher-order biofuels including higher alcohols, alkenes, and alkanes with longer carbon chains. However, biosynthesizing higher-order biofuels compounds (C > 6) has proven more difficult than synthesizing alcohols (C2-C5) using microorganisms as producing platform. This is largely because the techniques used to transform microbial metabolic pathways via genetic engineering are predominantly based on the Ehrlich pathway of the microorganism itself—namely, the α-keto acid pathway [21,22]. With this technique, different branched chain amino acids were converted into various kinds of alcohols (C2-C5) by importing a novel decarboxylation reduction pathway.

Thus, a problem arises when transforming microorganisms to produce higher-order biofuels. This problem requires specialized enzyme combinations and genetic engineering techniques for the optimization of metabolic capacity through functional reconstitution of long metabolic pathways. Specifically, the degree of difficulty or ease can vary widely because of the different complexities of diverse metabolic networks corresponding to the different genetic backgrounds of various microorganisms. Natural microorganisms undergo continuous, natural evolution, which allows for the development of both the best enzymatic system and specific synthetic route(s) in the synthesis of specific end-product for its own growth requirements. When viewed from an industrial perspective, there is currently no strain that could be used as a producer strain without manmade interventions to convert it to a highly productive strain suitable for large-scale industrialized applications. An ideal approach to achieve this objective would be to modify these microorganisms using a unique biosynthesis pathway for the generation of a specific chemical. This could be done either using native or non-native modified microorganisms as the starting host for subsequent engineering. A microorganism's synthetic capacity for the target product is primarily determined by its microbiology and physiology (Fig. 1). Different species have different cellular structures, different control mechanisms for various metabolic pathways, and different kinds of enzymes with their own respective cellular spatial distributions. For example, eukaryotes have a more complex cellular membrane structure than prokaryotes. This difference can have an important influence on metabolic mediation and control, such as determining ion concentration gradient(s) across the membrane, regulating inter- and intracellular material synthesis and transportation, and providing a barrier for the separation of different metabolic pathways [23–25]. Therefore, the synthetic cellular location and capabilities of synthetic pathways for the production of higher-order biofuels may not be equivalent between fungi and bacteria. Different microorganism species have different abilities for the production of target compounds because they have differential spectra for metabolic enzymes as well as differential resistance to the imbalances of metabolic flow. This variation typically results in different target product yields [11,26–30]. For instance, some biological engineering has been conducted to improve: cellular metabolism, genetic and cellular structure regulation such as control for cytosol or mitochondria, the transfer network for endogenous secondary metabolites, and metabolic flux in order to increase the concentration and/or productivity of the natural or non-natural biological products [31–33]. Thus, different microorganism hosts have different pathways, leading to design and program challenges as well as technical, operational, and workability difficulties. Therefore, selecting an appropriate strain for transformation as the starting host for later engineering is a very important step for biofuel production. This is particularly true for higher-order biofuels with long carbon chain (C > 6), as it is a criterion that will directly determine the probability of its successful use in industrial application.

In this paper, we first discuss the general strategies employed for selecting an appropriate microorganism as starting expression host to produce higher-order biofuels. We then present instances where these strategies can be applied, as well as provide insight into which strategy is optimal under which circumstances. We also particularly offer an in-depth discussion of existing research for selected strains designed to produce higher-order biofuels and examples of their recent applications. We then discuss potential microorganism hosts and offer insight into how they should be used to produce higher-order biofuels. Finally, we highlight the great promise presented by using microorganisms as a viable biosynthetic platform for the production of higher-order biofuels. We present this perspective using recent progression in the field of genetic engineering and the advent of the latest generational Crispr-Cas9 genome editing technique, used in this instance to overcome the complexities presented by the genetic metabolic background of microorganisms at more advanced levels.

2. Extension types of carbon chain in biosynthetic platforms

Currently, some microorganisms have been used as biosynthetic platform to efficiently manufacture both natural and non-natural biofuels through the combined use of computational tools with systems metabolic engineering derived from recent developments in techniques that can be used in genome, transcriptome and proteome sequencing, and metabolome and fluxome analysis in a high-throughput manner [34–40]. To allow for the production of higher-order biofuels, it is crucial that the exogenous metabolic pathways are imported into a majority of native microorganisms. This would allow for the extension