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Current Opinion in Biotechnology

Steps towards 'drop-in' biofuels: focusing on metabolic pathways

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The past decade has witnessed rapid advance in microbial production of 'drop-in' biofuels from renewable resources. Various biosynthetic pathways have been constructed to produce biofuels with diverse structures, and multiple metabolic engineering strategies have been developed to increase biofuel titers, yields, productivities and system robustness. In this review, we intend to give a brief but comprehensive overview of the most recent progresses on four essential pathways leading to 'drop-in' biofuel production, with an emphasis on the metabolic pathway efficiencies and biofuel structures. Furthermore, we also provide an insightful discussion on optimization strategies to improve the robustness of the microbial platforms for biofuel production.

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Current Opinion in Biotechnology 2018, **53**:26–32

This review comes from a themed issue on **Chemical biotechnology**

Edited by **Patrick Cirino** and **Mattheos Koffas**

http://dx.doi.org/10.1016/j.copbio.2017.10.010

Introduction

Since bioethanol and biodiesel were commercialized as the first-generation biofuels, the biofuel field has achieved tremendous advances on various scales from benchtop to large-scale reactors. To produce 'drop-in' biofuels on an industrial scale, several efforts have been made: the feedstock choices have been expanded from food crops (e.g. corns and oilseeds) to abundant and low-cost non-food biomass (e.g. corn stover and switchgrass) [1], solid wastes (e.g. industrial wastes and sewage sludge) [2], natural resources (syngas, methanol and methane) [3], and carbon dioxide [4]. Meanwhile, the fermentation host choices have expanded beyond the traditional model microorganisms such as *Escherichia coli* and *Saccharomyces cerevisiae* to a wide variety of non-model organisms. These new hosts variously feature unique capabilities in assimilating target feedstock, adapting to specialized fermentation conditions, and producing target biofuels in high efficiencies.

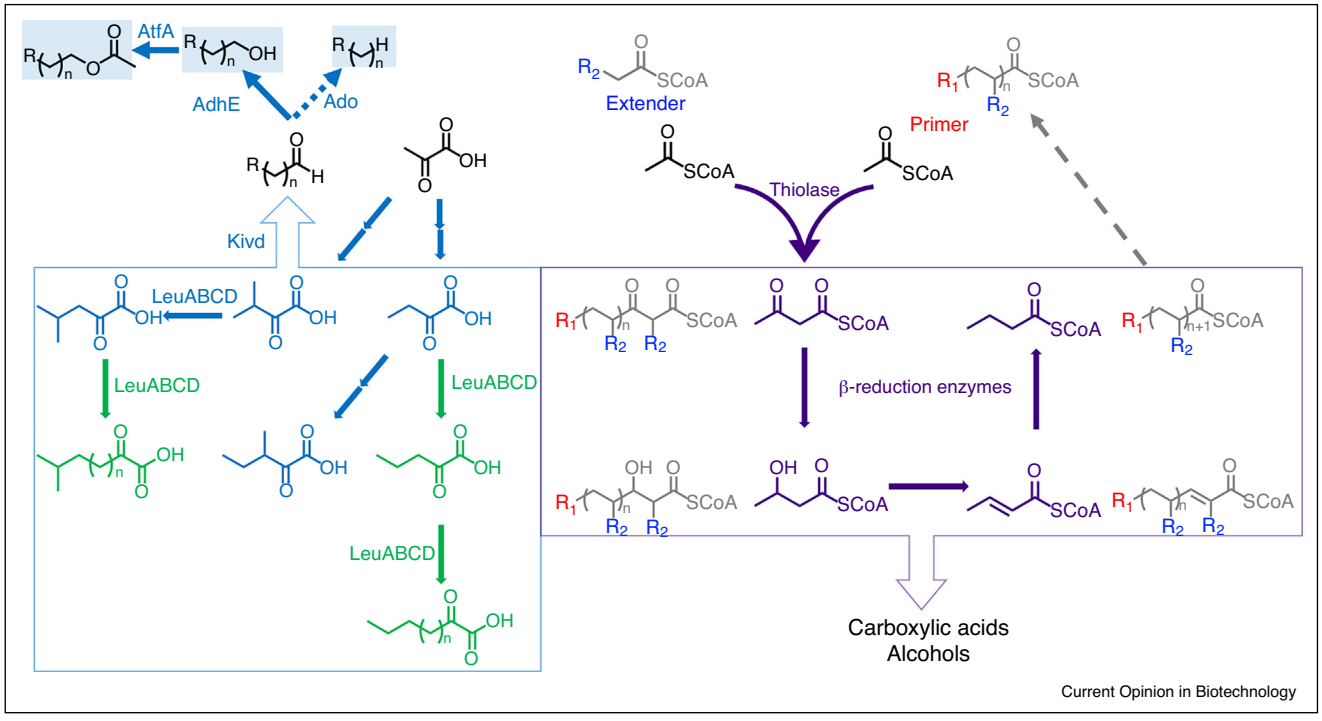
Meanwhile, by engineering different biosynthetic pathways, significant progress has been made in diversifying the structures of the bioproduced fuels, so that their physiochemical and combustion properties match with conventional engines and transportation infrastructures [5]. Most of these 'drop-in' biofuels are derived from five landmark pathways, including the α-keto acid pathway, the non-decarboxylative Claisen condensation pathway, the fatty acid pathway, the isoprenoid pathway, and the polyketides pathway. Compared to conventional bioethanol and biodiesel, biofuels produced from these pathways often have properties closer to those of petroleum-based fuels, and they are compatible with spark-ignition, compression-ignition, and gas-turbine engines [6]. Because the polyketides pathway has been reviewed thoroughly elsewhere [7], this review will focus on progress from the other four pathways within the past three years, with an emphasis on the structures of pathway products and strategies to improve pathway efficiencies.

α-Keto acid pathway

Short-chain alcohols (including isobutanol, 3-methyl-1-butanol, and 2-methyl-1-butanol) were produced from the α-keto acid pathway by decarboxylation and reduction of cellular short-chain α-keto acids, intermediates of the branched-chain amino acid biosynthesis, using an α-keto acid decarboxylase and an alcohol dehydrogenase [8] (Figure 1, light blue). Furthermore, by altering the substrate specificity of LeuABCD enzymes, the acyl chain length of α-keto acids can be further increased by recursively condensing with acetyl-CoA and releasing CO_2. Using this strategy, alcohols with medium chain-lengths (up to C8) or with branched/aromatic terminals have been produced [9•] (Figure 1, green). Meanwhile, α-keto acids or their derived alcohols can also be converted to short-chain alkanes [10] and to acetate-based esters [11] for biofuel applications.

One advantage of the α-keto acid pathway is its ability to add one carbon atom at a time to the acyl-chain, allowing precise control over chain-length. However, this chain elongation process sacrifices carbon efficiency and accumulates the reducing cofactor NADH (Table 1), which might be the reason for the lower titer of medium-chain alcohols (C6–C8) compared to that of isobutanol.

Figure 1



Biofuels produced from the α-keto acid pathway (left) and non-decarboxylative Claisen condensation pathway (right). α-Keto acids can be converted to aldehydes by an α-keto acid decarboxylase (Kivd). Aldehydes can then be converted to alcohols by an aldehyde dehydrogenase (AdhE) or to alkanes by an aldehyde decarbonylase (colored blue). α-keto acids can be elongated by a LeuABCD mutant to yield longer chain α-keto acids (colored green). The natural-occurring non-decarboxylative Claisen condensation pathway is colored purple. The engineered primers and extenders used in the non-decarboxylative Claisen condensation pathway are colored grey.

Table 1

The carbon efficiencies, cofactor balances, and highest titers produced from four pathways

Pathways	Products	Carbon recovery from glucose	Reducing cofactor imbalance	Highest titer in *E. coli*
α-Keto acid pathway	1-Butanol	44.4%	+ 6 NADH	1 g/L [12]
	Isobutanol	66.7%	+ 1 NADH, − 1 NADPH	22 g/L [8]
	3-Methyl-1-butanol	55.6%	+ 4 NADH, − 1 NADPH	4.4 g/L [13]
	2-Methyl-1-butanol	83.3%	+ 1 NADH, − 3 NADPH	1.25 g/L [14]
	1-Pentanol	41.7%	+ 8 NADH	4.3 g/L [15]
	1-Hexanol	40.0%	+ 10 NADH	302 mg/L [9•]
	1-Heptanol	38.9%	+ 12 NADH	80 mg/L [9•]
	1-Octanol	38.1%	+ 14 NADH	2.0 mg/L [9•]
Fatty acid pathway	Fatty acids (C14–C18)	66.7%	+ 14 NADH, − 12 NADPH (C14)	21.5 g/L [16••]
	Fatty alcohols (C14–C18)	66.7%	+ 12 NADH, − 12 NADPH (C14)	3.82 g/L [17]
	Alkanes (C9–C13)	60–61.9%	+ 13 NADH, − 12 NADPH (C13)	580 mg/L [18]
Non-decarboxylative Claisen condensation	1-Butanol	66.7%	0	30 g/L [19]
	Fatty acids (C6–C10)	66.7%	+ 2 NADH (C8)	3.8 g/L [20]
MVA pathway	Limonene	55.6%	+ 8 NADH	1.35 g/L [21]
MEP pathway	Limonene	66.4%	0	35.8 mg/L [22]

Fatty acid pathway

Because oilseed-derived biodiesel suffers from inferior low-temperature performance and competes with food supply, engineering efforts have been made to overproduce microbial-based fatty acid-derived fuels. Fatty acids (FAs) are biosynthesized *in vivo* by either the discrete fatty acid synthase complex FASII (in most bacteria) or a multifunctional FASI synthase (in eukaryotic cells). Initiated by the condensation of acetyl-CoA with malonyl-ACP, the four-carbon acyl chain product is elongated on