Revisiting metabolic engineering strategies for microbial synthesis of oleochemicals

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Abstract:

Microbial production of oleochemicals from renewable feedstocks remains an attractive route to produce high-energy density, liquid transportation fuels and high-value chemical products. Metabolic engineering strategies have been applied to demonstrate production of a wide range of oleochemicals, including free fatty acids, fatty alcohols, esters, olefins, alkanes, ketones, and polyesters in both bacteria and yeast. The majority of these demonstrations synthesized products containing long-chain fatty acids. These successes motivated additional effort to produce analogous molecules comprised of medium-chain fatty acids, molecules that are less common in natural oils and therefore of higher commercial value. Substantial progress has been made towards producing a subset of these chemicals, but significant work remains for most. The other primary challenge to producing oleochemicals in microbes is improving the performance, in terms of yield, rate, and titer, of biocatalysts such that economic large-scale processes are feasible. Common metabolic engineering strategies include blocking pathways that compete with synthesis of oleochemical building blocks and/or consume products, pulling flux through pathways by removing regulatory signals, pushing flux into biosynthesis by overexpressing rate-limiting enzymes, and engineering cells to tolerate the presence of oleochemical products. In this review, we describe the basic fundamentals of oleochemical synthesis and summarize advances since 2013 towards improving performance of heterotrophic microbial cell factories.

Highlights:

- Review of advances in microbial oleochemical production since 2013
- Overview of the structure and enzymology of oleochemical biosynthesis
- Summary of metabolic engineering strategies for improving oleochemical production
- Commentary on the state of oleochemical synthesis

Keywords: oleochemical; *Escherichia coli*; *Yarrowia lipolytica*, *Saccharomyces cerevisiae*; metabolic engineering; fatty acid; enzyme engineering; biodiesel; beta-oxidation; thioesterase

Abbreviations:

acyl-ACP reductase (AAR); acetyl-CoA carboxylase (ACC); acyl-CoA reductase (ACR); aldehyde deformylating oxidases (ADO); alka(e)nes (ALKs); carboxylic acid reductase (CAR); endoplasmic reticulum (ER); fatty acid ethyl-esters (FAEEs); fatty acid methyl-esters (FAMEs); fatty alcohols (FAOHs); methyl ketones (MKs); pyruvate decarboxylase (PDC); reversal beta-oxidation (r-BOX); S-adenosyl-L-methionine (SAM); triacylglycerols (TAGs); wax ester synthases/diacylglycerol acyltransferases (WS/DGAT); free fatty acid (FFA)

1. Introduction

Oleochemicals are defined as a class of aliphatic compounds industrially-derived from animal or vegetable lipids. Oleochemical products are classified according to the chain length, the terminal reductive state (e.g. acid, aldehyde, ester, olefin, alcohol, alkane), and any modifications (e.g. branching, unsaturation, hydroxyl-, cyclopropane-) to the main chain. These chemical features dictate the value and end uses of each oleochemical. For instance, long-chain fatty acids (C_{16}-C_{18}) have a long history of use as soaps while medium-chain fatty acids (C_8-C_{12}) have found additional uses as herbicides (Vaughn and Holser, 2007), precursors to lubricants, and polymer additives. Long-chain fatty acid methyl-esters (FAMEs) and fatty acid ethyl-esters (FAEEs) are the main components of commercial biodiesels that provide the energy for combustion. Introduction of branches in either the alcohol or acyl-portions of these esters decreases the cloud point of biodiesel, enabling superior use in cold-climates (Deng et al., 2015; Lee et al., 1995) . In addition to use as fuels, medium-chain FAMEs, FAEEs, and methyl ketones (MKs) are used as flavors and fragrances (Longo and Sanromán, 2006). Fatty alcohols (FAOH) are used to produce laundry detergents, industrial lubricants and surfactants, medicines, and personal care products. Long-chain alka(e)nes (ALKs) can be blended with liquid transportation fuels and serve as "drop in" replacements for petroleum-derived alkanes in fuel, solvent and other chemical applications. Long- and medium-chain α-olefins are used to produce detergents and plasticizers, and as monomers for elastomers used in automotive parts. In addition, very-long-chain oleochemicals such as fatty alcohols, e.g. 1-docosanol (C_{22}) and fatty waxes, e.g. Jojoba oil, are widely used in lubricants, detergents, polymers, photographic film-processing agents, coatings, cosmetics and pharmaceuticals (Jannin and Cuppok, 2013; Miwa, 1984; Taylor et al., 2010; Wisniak, 1994). These wide-ranging applications, particularly biodiesel, are driving continued growth of the oleochemical industry.

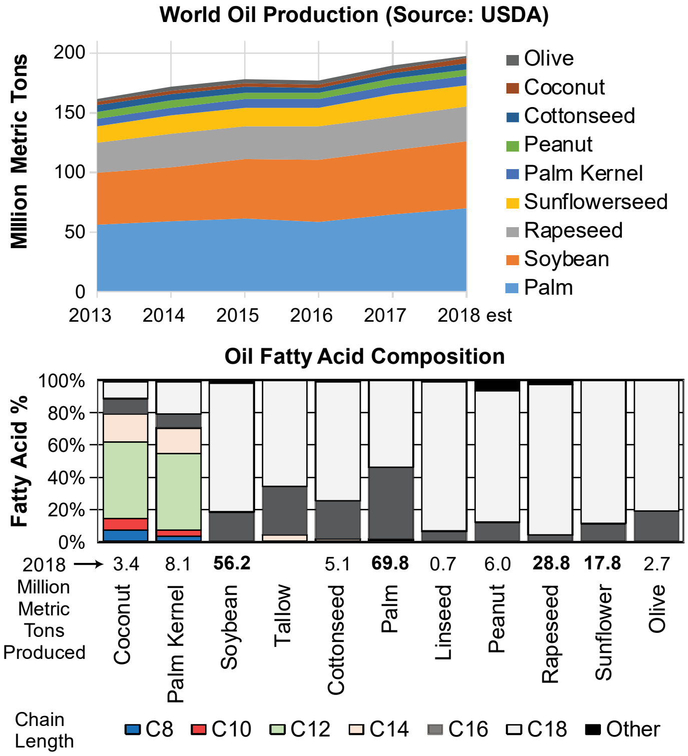

Fig. 1. Oleochemical Production Trends. Top: Production of vegetable oils has grown over the last five years (Source: USDA-ERS). Bottom: The composition (Harwood et al., 2007) of vegetable oils varies with most dominated by long chain (C_{16}-C_{22}) fatty acids. Only a handful of oil crops have high proportions of medium chain fatty acids.

As oleochemical demand has grown, so has production of vegetable oils from oil seed crops; world production of vegetable oils increased over 20% between 2013 and 2018 (Fig 1, USDA-ERS). The majority of fatty acids present in high-volume oils are classified as long chain ($\geq C_{16}$) (Harwood et al., 2007) leaving natural sources of medium chain lipids (C_6-C_{14}) in shorter supply (with the exception of dodecanoic acid which is common in both coconut and palm kernel oils). The lack of natural sources leaves room for metabolic engineering to play a role in generating these chain length lipids as well as other naturally uncommon oleochemicals from renewable and unrelated carbon sources. Over the past four decades, biochemists, microbiologists, and engineers have contributed greatly to discovering enzymes involved in oleochemical synthesis, unlocking key regulatory points that control fatty acid biosynthesis in model hosts, and demonstrating metabolic engineering strategies to produce specific oleochemical products (Fig. 2). These topics have been detailed in many past reviews (Cronan, 2003; d'Espaux et al., 2015; Lennen and Pfleger, 2013, 2012; Marella et al., 2018; Pfleger et al., 2015; Sarria et al., 2017; Zhou et al., 2018) and will be summarized herein.

The majority of oleochemicals sell for relatively low costs that are comparable to the costs of the sugar feedstocks used to make them. For this reason, oleochemical yield is the most critical parameter in evaluating strain performance. Unfortunately, most oleochemicals, with the exception of triacylglycerols (TAGs), are not common metabolic end-products. Therefore, it is not surprising that microbes capable of producing industrially relevant quantities (~100 g/L titers) at high yield (>90% of theoretical) have not been isolated from natural environments. Instead, the metabolism of common industrial microbes, e.g. *Escherichia coli*, *Saccharomyces cerevisiae*, and oleaginous microbes (TAG producers), e.g. *Yarrowia lipolytica*, *Rhodococcus opacus and Rhodosporidium toruloides*, have been altered to direct carbon flux from central metabolism towards lipid synthesis and the ultimate oleochemical product of interest. In some instances, metabolism can be engineered to produce oleochemicals as fermentation products (e.g. fatty alcohols via a thiolase-driven reversal of β-oxidation) (Dellomonaco et al., 2011; Mehrer et al., 2018) but the vast majority of oleochemicals are classified as secondary metabolites, i.e. metabolic products that compete with growth for cellular resources. The fact that maximum oleochemical yield occurs when the cell is directing zero carbon flux to biomass means that cells must work against the evolutionary pressure to grow in order to maximize yields of oleochemicals. Even for oleaginous organisms where nutrient limitations can be used to by-pass evolutionary pressures, the maximum lipid accumulation occurs when the minumum carbon flux is dedicated to biomass generation. These challenges have limited the number of compounds that have been produced in high yields, titers, and rates in microbial hosts. The remainder of this review will focus on the progress that has been made by the field after our last review (Pfleger et al., 2015) to improve the production of oleochemical products and close with a discussion of barriers that remain to be circumvented. The review focuses primarily on production of unmodified oleochemicals in heterotrophic species. Others have reviewed production of lipids in photosynthetic hosts (Klok et al., 2014; Remmers et al., 2018; Wijffels and Barbosa, 2010) and production of modified fatty acids (Jiang et al., 2018; Seo et al., 2015; Uemura, 2012; Werner and Zibek, 2017).

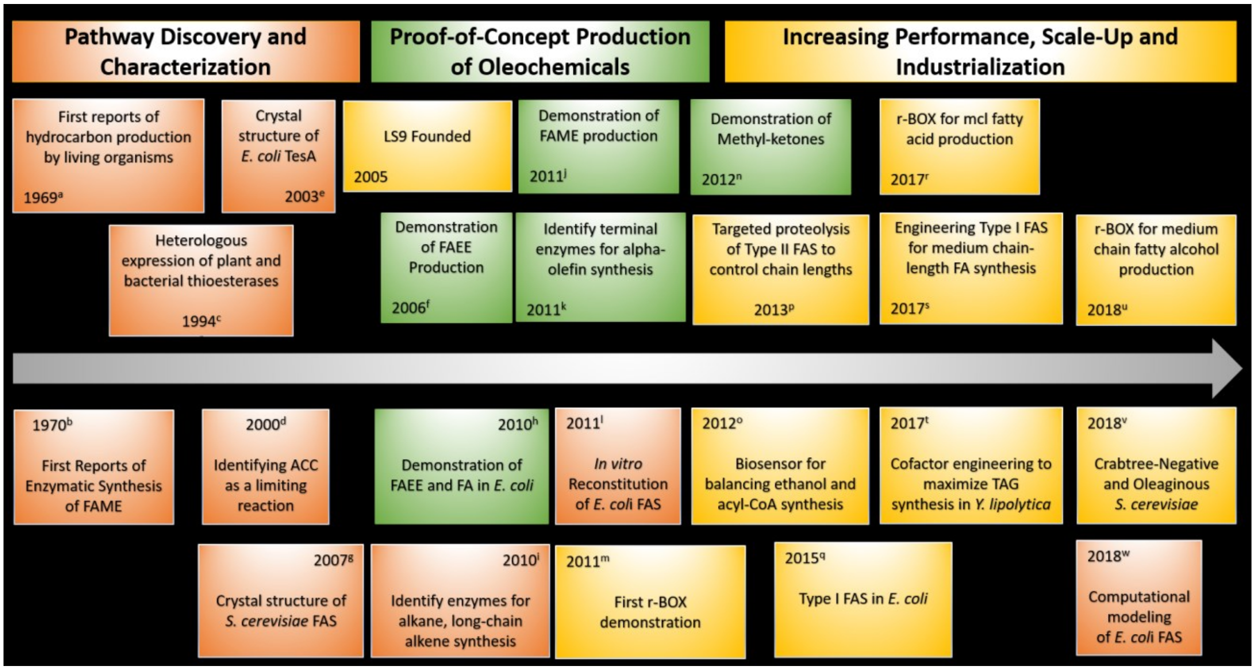

Fig. 2. Timeline of select achievements. References a-w are: [a](Winters et al., 1969); [b](Fehler and Light, 1970); [c](Voelker and Davies, 1994); [d](Mark S. Davis et al., 2000); [e](Lo et al., 2003); [f](R. Kalscheuer et al., 2006); [g](Lomakin et al., 2007); [h](Steen et al., 2010); [i](Schirmer et al., 2010) [j](Nawabi et al., 2011); [k](Mendez-Perez et al., 2011; Rude et al., 2011); [l](Yu et al., 2011); [m](Dellomonaco et al., 2011); [n](Goh et al., 2012); [o](Zhang et al., 2012a); [p](Torella et al., 2013); [q](Haushalter et al., 2015); [r](Wu et al., 2017a); [s](Gajewski et al., 2017); [t](Qiao et al., 2017) [u](Mehrer et al., 2018) [v](Yu et al., 2018) [w](Ruppe and Fox, 2018)

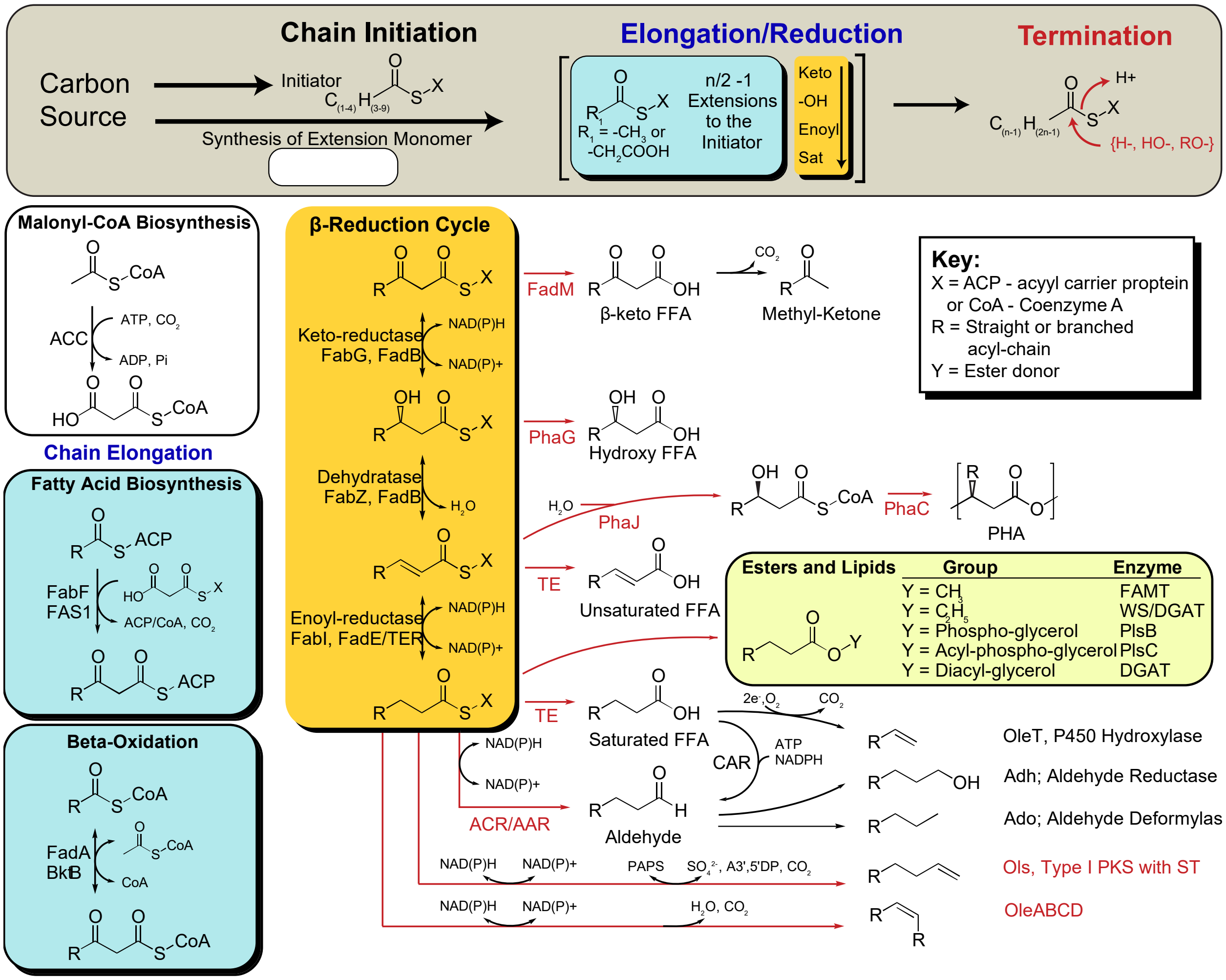

Fig. 3. Overview of Oleochemical Biosynthesis. Top. Oleochemicals are made in four phases, initiation, elongation/reduction, termination, modification (not shown). Carbon sources are fed through central metabolism to initiators (e.g. acetyl-CoA, propionyl-CoA, acetoacetyl-ACP) and extension monomers (e.g. acetyl-CoA, malonyl-CoA). The elongation-reduction cycle extends chains by two carbons and reduces (orange box) the beta-position to a saturated carbon. Elongation reactions (Blue boxes) are driven by fatty acid synthases or thiolases in most oleochemical pathways. Termination reactions (red arrows) act on various intermediates in the reduction cycle to produce FFA, aldehydes, esters, and olefins. Some of these products can be further converted by additional enzymes to olefins, alcohols, aldehydes, and PHA.

2. Fundamentals of fatty acid initiation, elongation and termination reactions

The alkyl chains of fatty acids, lipids, and oleochemicals are synthesized via an iterative elongation and reduction cycle that adds two carbons to the chain in each turn. The biochemistry of the individual biosynthesis pathways have been thoroughly reviewed elsewhere (Beld et al., 2015; Cronan, 2003; Heath and Rock, 2002; Lennen and Pfleger, 2012; Pfleger et al., 2015) and will therefore be briefly summarized here. There are many variations on how individual compounds are synthesized but in general, the biosynthetic pathway is comprised of four phases (Fig. 3): 1.) synthesis of building blocks (i.e. starter units, elongation units, ATP, reducing power), 2.) acyl-chain elongation and reduction, 3.) chain termination, and 4.) modification (omitted in Fig. 3 and not covered in this review). Substrates in the elongation, reduction, termination and modification phases are acyl-thioesters comprised of the acyl chain and either coenzyme A (CoA) or acyl-carrier protein (ACP) moieties (symbolized by S-X in Fig. 3). The CoA and ACP groups serve as molecular handles and influence the binding of biosynthetic enzymes to specific substrates (Beld et al., 2015; Chen et al., 2018; Meier and Burkart, 2009; Nguyen et al., 2014). The majority of natural oleochemicals are derived from even, straight-chain fatty acids where biosynthesis begins with an acetyl-CoA/ACP starter unit. More rarely, odd-chain (propionyl-CoA starter) and branched chain (starters are branched acyl-CoAs created in amino acid biosynthesis) fatty acids are naturally found and have been overproduced in heterologous hosts (Bentley et al., 2016; Cao et al., 2015; Tseng and Prather, 2012) where alternative initiation enzymes (e.g. FabH, BktB) synthesize the unusual starter units (Choi et al., 2000). As chains grow there is a competition between further chain elongation and termination reactions where the acyl chain is released from the thioester by hydrolysis, reductive cleavage, or transesterification. The relative kinetics of each reaction (i.e. the rate of elongation versus the rate of termination) dictate the distribution of final products. In eukaryotes, the enzymes that catalyze the elongation and reductive cycle are fused into a single polypeptide (e.g. mammalian Type I FAS) or two polypeptide (e.g. fungal Type I FAS) to facilitate the transfer of intermediates and channel synthesis towards the desired products (C_{16}-acyl-CoA) (Herbst et al., 2018; Lomakin et al., 2007). In contrast, most prokaryotes use a Type II system in which each enzyme is a distinct protein; a feature that has been exploited to alter the desired product profile using thioesterase enzymes capable of targeting medium chain acyl-ACPs.

In all pathways, the elongation reaction (Fig. 3 left) is either an irreversible decarboxylative Claisen condensation (e.g. FabF or FAS1) using malonyl-CoA/ACP as the donor or reversible thiolase-catalyzed Claisen condensation (e.g. FadA or TER) using acetyl-CoA as the donor. Malonyl-CoA is synthesized from acetyl-CoA, bicarbonate, and one ATP (Fig. 3, white box). The irreversible decarboxylation performed as part of the Claisen condensation provides a thermodynamic driving force at the expense of the extra ATP required to synthesize malonyl-CoA (Shen et al., 2011). The extra ATP cost reduces the theoretical yield of oleochemicals synthesized by a small (~5%) but significant amount when cells are cultivated aerobically; e.g. the theoretical yield of dodecanoic acid is 0.39 g/g via a thiolase pathway and 0.37 g/g via fatty acid biosynthesis (calculated from flux balance analysis of a modified iJO1366 *E. coli* genome scale reconstruction). The difference in theoretical yield is much larger when cells are grown anaerobically because of the reduced ATP synthesis achieved during fermentation; e.g. the theoretical yield of dodecanoic acid is 0.39 g/g via a thiolase pathway and 0.27 g/g via fatty acid biosynthesis (Mehrer et al., 2018). The reductive cycle (Fig 3. orange box) requires two reducing equivalents, often in the form of NADPH, per turn of the cycle. The NADPH requirement reduces theoretical oleochemical yields because of reduced ATP synthesis of pathways that produce NADPH from common sugars (e.g. pentose-phosphate pathway vs. glycolysis).

In effort to better understand flux control points, type II fatty acid synthesis was reconstituted *in vitro* using enzymes from both *E. coli* and other microbes (Kuo and Khosla, 2014; Xiao et al., 2013; Yu et al., 2011). From these studies, the field has learned that 1.) enoyl-ACP reductase (FabI^{EC}) and β-hydroxyacyl-ACP dehydratase (FabZ^{EC}) enhanced the rate of fatty acid production in a dose-dependent manner; 2.) β-ketoacyl-ACP synthases III (FabH^{EC}) and II (FabF^{EC}) decreased FAS activity at concentrations higher than 1 μM; 3.) the ratio of β-hydroxyacyl-ACP dehydratase (FabA^{EC}) and β-ketoacyl-ACP synthase I (FabB^{EC}) regulated the degree of unsaturated fatty acid biosynthesis in *E. coli*; 4.) FabH was the limiting enzyme in cyanobacteria (Heath and Rock, 1995; Yu et al., 2011). Recently, a detailed mechanistic kinetic model of type II fatty acid biosynthesis was constructed. The model's perturbation analysis supported these findings and showed: 1) overexpression of FabH^{EC} inhibited fatty acid synthesis by depleting malonyl-ACP pool; 2) overexpression of holo-ACP, fatty acid synthase (FabF^{EC}), and thioesterase (TesA'^{EC}) inhibited fatty acid synthesis by sequestering components; 3) overexpression of FabI^{EC} and FabZ^{EC} enhanced fatty acid synthesis rates. Analysis of free fatty acid (FFA) composition produced by using the model indicated: 1) changes in the ratios of FAS components altered the average length of fatty acids and 2) ketoacyl synthases and thioesterases were required for narrow product profiles (Ruppe and Fox, 2018). Given the success of these *in vitro* studies, further exploration of the enzymes in other organisms may shed light on the key biochemical and regulatory differences between hosts, thereby guiding further metabolic engineering.

The biochemistry of the enzymes involved in chain termination has been reviewed elsewhere (Beld et al., 2015; Fillet and Adrio, 2016; Kang and Nielsen, 2017; Lennen and Pfleger, 2013; Sarria et al., 2017) and will be briefly summarized here. Termination reactions branch from each compound in the reductive cycle (**Fig. 3** red arrows) with long-chain products being more common. Fatty acid biosynthesis supports two main roles in the cell – creation of membranes and cell wall components and storage of carbon in energy dense granules. Membrane lipids, storage lipids, and waxes are made by transesterifying saturated and unsaturated acyl-ACP/CoAs onto phosphoglycerol, acyl-glycerols (Cronan, 2003) or fatty alcohols respectively (Rontani, 2010). These reactions are catalyzed by acyltransferases with widely varying substrate specificity to produce medium-chain, long-chain, and very long-chain ($> C_{20}$) products. Fatty acid ethyl-esters (FAEEs) are synthesized by wax ester synthases/diacylglycerol acyltransferases (WS/DGAT) that have activity on shorter chain alcohols (e.g. ethanol) (Rainer Kalscheuer et al., 2006). The most common form of biodiesel, fatty acid methyl esters (FAMEs), can be enzymatically synthesized through direct methylation of fatty acids by a methyltransferase that uses S-adenosyl-L-methionine (SAM) as methyl group donor (Nawabi et al., 2011). In addition to lipid oils, microbes can store fatty acids in the form of polyhydroxyalkanoates (PHAs), polymers of β-hydroxy fatty acids. Here, acyl-CoAs are converted to (3R)-hydroxyacyl-CoA's (via hydration of enoyl-CoAs by PhaJ) and polymerized by variants of PHA synthases (PhaC) that accept long chains (Agnew and Pfleger, 2013). The monomers are produced either from transesterification of acyl-ACPs or incorporation of free fatty acids via partial turns of β-oxidation (Agnew et al., 2012).

Acyl-thioester reductases catalyze the reductive cleavage of acyl-ACPs (acyl-ACP reductase – AAR) and acyl-CoAs (acyl-CoA reductase – ACR) to yield fatty aldehydes. The reductive power typically comes from oxidation of NADPH. Fatty aldehydes can also be produced by carboxylic acid reductase (CAR), an enzyme that activates a free fatty acid with ATP hydrolysis, covalently links the fatty acid to the enzyme, and performs a similar reductive hydrolysis reaction to ACR/AARs. Fatty aldehydes can be further reduced to fatty alcohols (via promiscuous aldehyde reductases) or converted to alkanes by aldehyde deformylating oxidases (ADO) that use oxygen and a protein reductive system (NADPH, ferredoxin, ferredoxin reductase). Fatty alcohols are widely synthesized to produce wax esters of fatty alcohols and fatty acids for use in cell walls and as energy storage (Rontani, 2010). Some species use multi-domain enzymes that catalyze reduction of both acyl-thioesters and fatty aldehydes from the same polypeptide (Willis et al., 2011). The alkane synthesis pathway was first identified in cyanobacteria according to a bioinformatics comparison of alkanes producing and non-producing strains. It is generally accepted that the second step (ADO) is rate-limiting due to its slow-turnover of fatty aldehydes – a potentially toxic species that further complicates use of this pathway.

In addition to saturated alkanes, some microbes produce olefins. All sequenced cyanobacteria genomes encode either the AAR/ADO pathway for producing alkanes or a pathway for producing α-olefins (Coates et al., 2014). The presence of either compound is required for modulating photosynthetic electron flow at low temperatures (Berla et al., 2015; Knoot and Pakrasi, 2019). The biosynthesis of α-olefins is catalyzed by a polyketide synthase-like protein, Ols, first characterized in *Synechococcus sp*. strain PCC (Mendez-Perez et al., 2011) 7002 or a cytochrome P_{450} enzyme OleT from *Jeotgalicoccus* sp. (Rude et al., 2011). Ols uses saturated and unsaturated C_{18}-acyl-ACPs as a substrate to produce 1-nonadecene and 1,14-nonadecadiene via keto-synthase, β-ketoreductase, sulfotransferase, desulfation, and decarboxylation reactions catalyzed by the multi-domain enzyme. The OleT enzyme is a fatty acid decarboxylase that converts long-chain fatty acids to C_{19} and C_{21} α-olefins. Two novel enzymes that catalyze similar reactions have been more recently discovered and characterized. A family of oxidases, UndA, conserved in *Pseudomonas* sp. are responsible for biosynthesis of α-olefins using an oxygen-activating, non-heme iron-dependent mechanism (Rui et al., 2014). A family of desaturase-like enzyme, UndB, is responsible for the biosynthesis of α-olefin through an β-hydrogen abstraction (Rui et al., 2015). In addition to α-olefins, very-long chain olefins and ketones are synthesized by condensing two acyl-chains to form a new bond between the carbonyl of one chain and the alpha carbon of the second (catalyzed by OleA of *Shewanella* species) (Sukovich et al., 2010). This intermediate undergoes keto-reduction, decarboxylation, and dehydration reactions (catalyzed by OleBCD) to yield the final olefin product. Olefins are widely used in many material applications and therefore these enzymes are attractive catalysts for biological production of high-value compounds.

The most commonly studied termination reaction is the hydrolysis of acyl-thioesters by thioesterases to produce free saturated, unsaturated, β-hydroxyl-, and β-keto-fatty acids; the latter of which can be enzymatically or chemically decarboxylated to yield methyl-ketones (Goh et al., 2012). In *E. coli* and *S. cerevisiae*, if fatty acid catabolism is blocked (e.g. deletion of acyl-CoA/ACP synthesis and/or β-oxidation) and an exporter is expressed, free fatty acids accumulate in the extracellular space (M S Davis et al., 2000; Hu et al., 2018; Lennen et al., 2010; Lu et al., 2008; Voelker and Davies, 1994). This strategy is particularly effective in *E. coli* because long-chain acyl-ACP, the key regulatory compound, is depleted in the presence of an active thioesterase (Jiang and Cronan, 1994). Some thioesterases have broad substrate specificity and act on many acyl-thioester chains (Cantu et al., 2011). Others, particularly those from plants, are specific for particular chain-lengths that often correspond to the major fatty acids present in their storage lipids (Cantu et al., 2011). In addition to making free fatty acids, thioesterases are also used to produce pools of specific length acyl-chains that are reactivated to acyl-CoAs, oxidized to the required reduction state, and processed by other termination enzymes to produce esters, alcohols, alkanes, olefins, polyhydroxyalkanoates, or methyl-ketones (Agnew et al., 2012; Akhtar et al., 2015; Goh et al., 2014; Steen et al., 2010; Youngquist et al., 2013). This strategy requires expression of an acyl-CoA synthetase (e.g. FadD), blockage of β-oxidation at the correct point in the oxidation cycle, and expression of the desired termination enzyme (**Fig. 4**). The motivation for this approach is the fact that most termination enzymes, except thioesterases, have broad substrate specificity. Here, the approach generates a pool of the desired chain-length acyl-CoA, such that the profile of oleochemical products matches that of the thioesterase used. The cost of this strategy is the extra ATP required for FFA reactivation by CAR or acyl-CoA synthases and potential futile cycles created by thioesterase activity on acyl-CoAs.

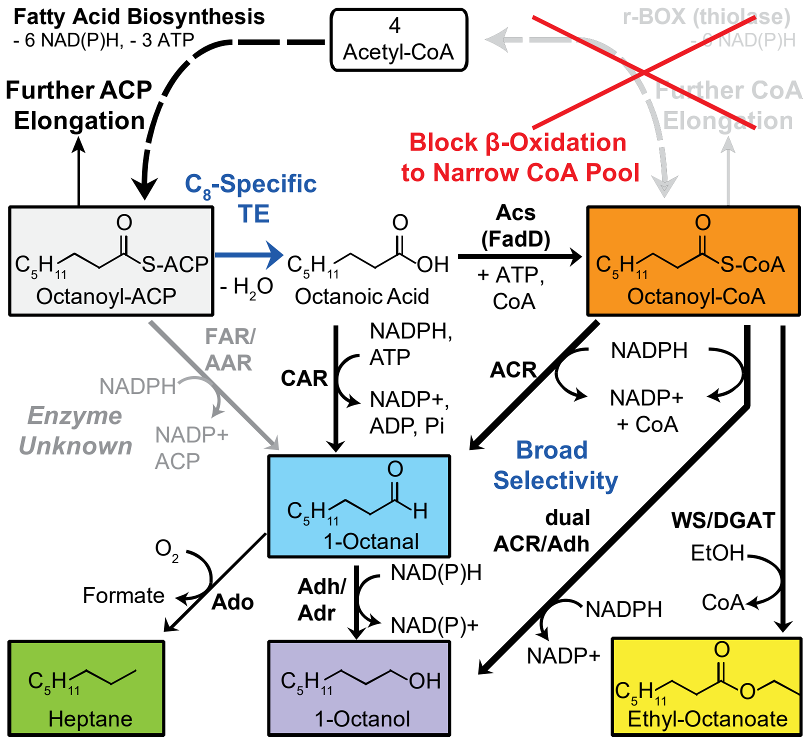

Fig. 4 – Strategy for selectively producing medium chain oleochemicals using thioesterases. Most termination enzymes have activity on a broad range of substrates. Consequently, few enzymes are capable of producing a specific termination product (e.g. octanol from octanoyl-ACP/CoA). Instead, thioesterases that act on a small set of substrates can be used to create a narrow

pool of free fatty acids. If β-oxidation is blocked, then the fatty acid pool can be converted to a narrow set of acyl-CoAs by acyl-CoA synthetases (Acs). These narrow pools can then be converted to specific oleochemicals by enzymes having broad substrate specificity acting on the most abundant acyl-thioester present. Unfortunately, the required blockage of β-oxidation prevents the r-BOX pathway from being used to produce the acyl-chains.

3. Engineering strategies to increase oleochemical production and chain-length selection

Maximizing flux through fatty acid biosynthesis is achieved by combining general metabolic engineering strategies: 1.) blocking pathways that compete with synthesis of key precursors or consume oleochemical products, 2.) pushing flux into fatty acid biosynthesis by overexpressing rate-limiting enzymes or enzymes that produce intermediates dedicated to fatty acid biosynthesis (e.g. malonyl-CoA), 3.) pulling flux through fatty acid biosynthesis by overexpressing enzymes and/or regulatory proteins that by-pass native regulation, 4.) balancing synthesis of required co-factors with building blocks and other cellular requirements, 5.) expressing auxiliary proteins that alleviate stresses and other problems caused by producing specific oleochemicals (Royce et al., 2015; Xu et al., 2017). In addition, maximizing particular chain-length oleochemical production can be achieved by altering key endogenous enzyme activities (e.g. thioesterase in *E. coli* and Type I FAS in yeast) or termination enzyme activities. Over the past five years, many of these efforts have increased production of oleochemicals in *S. cerevisiae*, *E. coli*, and oleaginous microbes. Progress is summarized in **Table 1**.

3.1. Increasing acetyl-CoA pool

Acetyl-CoA is the key building block of all oleochemicals and a central metabolite involved in many biochemical systems. This section briefly summarizes strategies implemented to increase the acetyl-CoA pool in *E. coli*, *S. cerevisiae* and *Y. lipolytica*. More comprehensive reviews on balancing intracellular acetyl-CoA pools can be found elsewhere (Krivoruchko et al., 2015; Nielsen, 2014; Pietrocola et al., 2015). The synthesis of acetyl-CoA is highly regulated and completes with production of several fermentation products, e.g. acetate, ethanol, lactate. In *E. coli*, deletion of these competing pathways is essential to direct flux through the reversed β-oxidation pathway (r-BOX) (Dellomonaco et al., 2011; Mehrer et al., 2018; Wu et al., 2017a) and reduce carbon flux to acetate that is used as part of the acetate switch (Wolfe, 2005) during rapid exponential growth. Unfortunately, deletion of the pathways that compete with acetyl-CoA synthesis often leads to reduced growth rates and/or substrate uptake rates. This is best exemplified in *S. cerevisiae* where ethanol production is eliminated by disruption of the three pyruvate decarboxylase (PDC) isozymes (Y. Chen et al., 2015; Dai et al., 2018; Oud et al., 2012). Triple PDC mutants grow very slowly on glucose and accumulate mutations that result in reduced glucose uptake. To overcome these challenges, Zhou et al. overexpressed a chimeric ATP:citrate lyase-malic enzyme-malate dehydrogenase-citrate transporter Ctp1, resulting in a 20% increase in fatty acid production. In addition, an engineered *S. cerevisiae* carrying deletions of fatty acyl-CoA synthases FAA1 and FAA4, and fatty acyl-CoA oxidase HFD1, overexpressing *R. toruloides* FAS, TesA' and ACC1 enabled 10.4 g/L free FFAs in fed-batch fermentation (Zhou et al., 2016). A separate study that integrated metabolic engineering and adaptive laboratory evolution converted *S. cerevisiae* to a Crabtree negative yeast (Dai et al., 2018), in the process eliminating ethanol production. Further development led to a strain capable of producing 33.4 g/L FFAs, representing a 4-fold improvement over a starting strain in a glucose limited and nitrogen restricted fed-batch cultivation (Yu et al., 2018). In contrast, the oleaginous yeast *Y. lipolytica* naturally generates a large flux to cytosolic acetyl-CoA, particularly under nitrogen starvation when storage lipids are accumulated. In effort to decouple acetyl-CoA flux from nitrogen starvation, and thereby shorten cultivation time, Xu et al investigated five alternative cytosolic acetyl-CoA pathways in *Y. lipolytica* (Xu et al., 2016). Overexpression a carnitine acetyltransferase Cat2 and a mitochondrial carnitine acetyltransferase Yat1 helped pull mitochondrial acetyl-CoA into cytosol and conferred the most improvement of lipid titer (~75%). Increasing the acetyl-CoA pool has been shown to be beneficial for increasing oleochemical production in both non-oleaginous and oleaginous organisms.

3.2. Increasing malonyl-CoA pool

Malonyl-CoA, the elongation unit used in fatty acid biosynthesis, is produced from acetyl-CoA by acetyl-CoA carboxylase (ACC), a rate-limiting reaction in fatty acid biosynthesis in several organisms. Efforts to increase malonyl-CoA pools have been detailed in prior reviews (Johnson et al., 2017; Pfleger et al., 2015; Sheng and Feng, 2015), so recent progress will be briefly summarized in this section. Balanced overexpression of ACC subunits increases production of free fatty acids in *E. coli* that co-express a thioesterase (M S Davis et al., 2000; Lennen et al., 2010). In yeast, several strategies increased malonyl-CoA production including 1.) overexpression of ACC1 by chromosomally swapping to a strong constitutive promoter (Qiao et al., 2015), 2.) overexpression of a mutated ACC1** (Ser659Ala, Ser1157Ala) that abolishes post-translational phosphorylation inhibition (d'Espaux et al., 2017)(Shi et al., 2014), 3.) by-passing the ACC pathway by expressing methyl malonyl-CoA carboxyltransferase and phosphoenolpyruvate carboxylase (Shin and Lee, 2017), 4.) overcoming a allosteric feedback inhibition by C_{16}-C_{20} saturated acyl-CoAs through overexpressing Δ-9 stearoyl-CoA desaturase in *Y. lipolytica* (Qiao et al., 2015), or through overexpressing a Δ9-desaturase (OLE1) in *S. cerevisiae* to increase membrane fluidity and the fatty acyl-CoA pool (d'Espaux et al., 2017). In addition, Liu et al. found that a mutant Mga2p regulator in *Y. lipolytica* led to increased unsaturated fatty acid biosynthesis and lipid accumulation, possibly due to reduced feedback inhibition of ACC (L. Liu et al., 2015). An alternative strategy is to balance malonyl-CoA production and consumption using a malonyl-

CoA responsive biosensor (Xu et al., 2014). In addition to this direct sensing strategy, indirect malonyl-CoA biosensors have been produced in *E. coli*, *Pseudomonas putida*, and *Corynebacterium glutamicum* using RppA, a type III polyketide synthase that synthesizes flaviolin, a red-colored pigment, from malonyl-CoA. The sensor was applied to identify high malonyl-CoA producers using a genome-wide sRNA knockdown library (Yang et al., 2018).

3.3. Leveraging β-oxidation as a high-yielding synthetic pathway

In a seminal work, Dellomonaco *et al.*, showed that β-oxidation enzymes can be used in a synthetic direction (r-BOX) to produce medium and long-chain oleochemicals via acyl-CoAs. The advantage of this pathway is the larger theoretical yields that can be achieved by avoiding ATP consumption required for malonyl-CoA synthesis. Since the first description in 2011, several groups have expanded the r-BOX concept to improve oleochemical production in bacteria and yeast (Kallscheuer et al., 2017). One study demonstrated that *E. coli* type II FAS can be used to comprise the pathway (Clomburg et al., 2018). A second explored the relative expression of key component enzymes in producing medium chain fatty acids (Wu et al., 2017a). The same group showed that increasing NADH availability is critical to boosting production of medium chain fatty acids from r-BOX in *E. coli* (Wu et al., 2017b). The r-BOX pathway is ideally suited for producing fatty alcohols because the biosynthesis pathway is redox balanced to glucose catabolism. This strategy led to the production of a distribution of medium chain fatty alcohols (~1.8 g/L) in *E. coli* (Mehrer et al., 2018). The r-BOX pathway has been assembled in the cytosol of *S. cerevisiae* to synthesize butanol, medium chain fatty acids and ethyl esters (Lian and Zhao, 2015). One main hurdle of developing r-BOX in yeast is the compartmentalization of β-oxidation pathway in the peroxisome away from pyruvate dehydrogenase (acetyl-CoA synthesis) in the cytosol. In this sense, two locations of r-BOX can be chosen: peroxisome or cytosol. To use the r-BOX pathway in the peroxisome one must increase the peroxisomal acetyl-CoA pool. Alternatively, the requisite r-BOX enzymes will need to be expressed in the cytosol along with strategies for increasing flux to acetyl-CoA.

The key enzyme in the r-BOX pathway is the reversible thiolase that elongates the acyl-chains. Specific thiolase variants have different substrate preferences and therefore help determine the ultimate product chain-length. The original description of the r-BOX pathway utilized a short-chain acyl-CoA transferase, YqeF, leading to accumulation of C_4 acyl-CoA and butanol. *Ralstonia eutropha* BktB has been widely used in r-BOX supporting synthesis of acyl-CoA of up to 10 carbons. For instance, in combination with a ‘TesA^{EC} can lead to 1.3 g/L C_6-C_{10} fatty acids (Kim et al., 2015) and in combination with a longer-chain acyl-CoAs thioesterase FadM led to 2.1 g/L C_{10} fatty acids (Kim and Gonzalez, 2018). Utilization of a long-chain acyl-CoA transferase FadA led to accumulation of longer chain-length CoA precursors (Dellomonaco et al., 2011; Mehrer et al., 2018). In addition, utilization of the specificity of the thiolase (BktB), reductase (PhaB), dehydratase (PhaJ) and enoyl-CoA reductase (Ter) allows accumulation of C_4 and C_6 acyl-CoA substrates, in combination with a broad-specificity carboxylic acid reductase and a cyanobacterial aldehyde decarbonylase led to 1.6 mg/L pentane (Sheppard et al., 2016). Efforts to engineer the specificity of thiolases have been reported (Bonk et al., 2018) and further engineering will likely be necessary to produce narrower product distributions.

3.4. Balance cofactors

Reducing power required for synthesis of many intracellular metabolites is often provided in the form of NADPH, and in the case of oleochemicals, NADPH is required in each elongation cycle and frequently in terminal reductions to convert acids into aldehydes, alcohols, olefins and alkanes. The NADPH requires balancing because catabolic pathways typically generate excess

NADH as electron carriers rather than NADPH. Efforts to redirect flux to balance the NADPH supply and demand have been guided by genome-scale metabolic modeling (Ranganathan et al., 2012; Tee et al., 2014). Alternatively, synthetic pathways can be comprised of natural variants of the reductive enzymes that prefer NADH or engineered variants that utilize NADH at higher rates (Javidpour et al., 2014). In *Y. lipolytica*, ^{13}C metabolic flux analysis identified the oxidative pentose phosphate pathway as the primary source of NADPH for lipid synthesis (Wasylenko et al., 2015). Similarly, a constraint-based model identified fluxes in a lower portion of glycolysis and pentose phosphate pathway are highly correlated to alkane synthesis in *E. coli*, due to supplementing reducing equivalent NADPH (Fatma et al., 2018). In *E. coli*, PPP flux was enhanced by overexpressing G6P dehydrogenase Zwf, eliminating ED pathway fluxes usage by deleting phosphogluconate dehydratase Edd, and eliminating reverse fluxes toward PEP by deleting PpsA and PflB (Fatma et al., 2018). A recombinant strain led to 2.54 g/L alkane production in a fed-batch fermentation. Alternatively, introduction of the Entner-Doudoroff pathway from *Zymomonas mobilis* into *E. coli* increased the NADPH regeneration rate by 25-fold (Ng et al., 2015). In *S. cerevisiae*, NADPH synthesis was enhanced by replacing PGI1 with ZWF1 to force flux through the pentose phosphate pathway, and deleting GDH1, a NADPH consuming pathway. The resulting strain produced 6 g/L of fatty alcohols with yield of 0.058 g/g glucose from a lignocellulosic hydrolysate (d'Espaux et al., 2017). Alternatively, NADH-producing enzymes have been replaced by NADPH producing variants, e.g. NADP+-dependent glyceraldehyde 3-phosphate dehydrogenase from *Clostridium acetobutylicum*, malic enzyme from *Mucor circinelloides* where the resulting *Y. lipolytica* strain produced a highest reported titer, 99 g/L lipids with 1.2 g/L/h productivity and 0.269 FAME g/g glucose (Qiao et al., 2017).

3.5. Controlling the chain length of oleochemicals

As discussed above, the majority of termination enzymes, with the exception of thioesterases, have poor selectivity in terms of activity on different chain-length products. As such, the product profile mirrors the distribution of acyl-thioesters that accumulate in the cell. For this reason, metabolic and protein engineering strategies have been applied to alter the chain length of acyl-thioesters produced by cells or to alter the selectivity/activity of termination enzymes to target specific substrates. In type II fatty acid biosynthesis, different β-ketoacyl synthase variants are used to extend ranges of acyl-thioester intermediates. For instance, in *E. coli*, FabH initiates the elongation cycle, FabF elongates medium chain ACPs, and FabB is essential for elongating unsaturated C_{10} and long chain acyl-ACPs. The overlapping specificity provides functional redundancy (i.e. only FabB is essential) and opportunities for altering the distribution of acyl-ACPs. In one such study, an *E. coli* FabF* variant incapable of extending C_8 acyl-ACPs was co-expressed with a FabB variant tagged with an inducible protease recognition sequence. Upon degradation of FabB, a large pool of C_8-ACP was created, resulting in increased flux to octanoic acid via a C_8 specific thioesterase (Torella et al., 2013). Computational models of fatty acid biosynthesis predict that the ratio of TesA (thioesterase) to FabF determines the mean fatty acid chain-length, and the relative substrate specificity of the thioesterase and β-ketoacyl synthase dictate the width of fatty acid profiles (Ruppe and Fox, 2018). These findings motivate protein engineering to alter the substrate preference of other termination enzymes.

In type II systems, termination enzymes can compete with β-ketoacyl synthases for free acyl-ACP substrates. In contrast, tethering of acyl-chains to the synthases in type I systems reduces access of trans-acting termination enzymes. For this reason, type I variants were created to replace the native termination domain (MPT) with medium-chain targeting thioesterase or methyl ketone synthase. The resulting enzyme in increased production of medium chain fatty acids or methyl ketones (Xu et al., 2016; Zhu et al., 2017b). Alternatively, the active sites of the synthase (KS), loading (acyltransferase - AT), and termination (MPT) domains were mutated to manipulate the chain length distribution of fatty acids synthesized (Gajewski et al., 2017; Rigouin et al., 2017). For instance, In the KS domain, M1251 protrudes into the KS binding channel and dividing the binding channel into an outer and inner volume. On acyl binding, the methionine rotates and gives access to the inner cavity. The neighboring G1250 is essential for the conformational changes of M1251. Variants comprised of G1250S and M1251W mutations increased C_6- and C_8-FFA by 12- and 56-fold over wild-type, respectively. The mutation of I306A of AT domain broadened substrate specificities. Together, a combinational variant (I306A, G1250S, M1251W) increased production to 646 mg/L C_6-C_8 FFAs.

3.6. Improving production of specific oleochemical products

Free fatty acids. The substrate profile of thioesterases can be broad or narrow and variants that target desirable chain lengths have been found in both plants and microbes (Cantu et al., 2011), albeit with varying activity in heterologous hosts. For this reason, protein engineering has been applied to increase thioesterase activity and selectivity towards desired chains. Thioesterase activity has been enhanced by altering the protein surface to better recognize *E. coli* ACP instead of the surface of the ACP found in the host from which the thioesterase was sourced (Sarria et al., 2018). A structure-guided mutagenesis algorithm was applied to the *E. coli* thioesterase TesA' (PDB 1U8U) to increase its activity towards medium chain acyl-ACPs. One variant, TesA' M141L, Y145K, L146K, produced a product profile with 50% C_8 while increasing the overall activity (Grisewood et al., 2017). Highly active variants of the *Cuphea palustris* FatB (native C_8 specificity >90%) were isolated from a random mutagenesis library using an octanoic acid selection (Hernández Lozada et al., 2018). The best variant (*Cp*FatB N28S, I65M, N-terminal truncation) increased the v_{max} by 15-fold enabling production of nearly 3 g/L octanoic acid from a single chromosomal copy of the gene (Hernández Lozada et al., 2018). As additional enzymes are crystalized, structure-guided mutagenesis algorithms are likely to play an important role in in providing access to enzymes capable of producing medium-chain length oleochemicals.

Fatty alcohols. Recently, homologues of dual function acyl-thioester reductases have been explored for varying the chain-length profile of fatty alcohols (Hofvander et al., 2011; Willis et al., 2011). Overexpression of *Marinobacter aquaeolei* VT8 ACR in *E. coli* led to 1.6 g/L C_{12} and C_{16} alcohols, with dodecanol coming from expression of a C_{12}-specific thioesterase (Youngquist et al., 2013). Overexpression of a dual function alcohol dehydrogenase YiaY led to 0.42 g/L C_6-C_{10} alcohols in an engineered *E. coli* r-BOX strain (Dellomonaco et al., 2011). Expression of FAR in yeast produced longer chain-length fatty alcohols: 1.) *Tyto alba* acyl-CoA reductase (*Ta*ACR) in *Y. lipolytica* led to 690.21 mg/L C_{16} and C_{18} alcohols (Wang et al., 2016); 2.) localization of *Ta*ACR in *S. cerevisiae* peroxisome produced C_{10} and C_{12} alcohols (Sheng et al., 2016); 3.) *Mus musculus* ACR in *S. cerevisiae* WRY1 led to 98.0 mg/L C_{16} and C_{18} alcohols (Runguphan and Keasling, 2014); 4.) *Mus musculus* ACR in *S. cerevisiae* yL434 produced 6.0 g/L C_{16} and C_{18} alcohols (d'Espaux et al., 2017). In each of these studies, the ACR itself was not used to dictate the product profile, indicating that ACR selectivity remains an open area for future study.

Alka(e)nes. In the first demonstration of heterologous alkane biosynthesis, expression of the ADO and AAR encoded by PCC7942_orf1593 and PCC7942_orf1594 in *E. coli* MG1655 led to accumulation of ~150 mg/L C_{13}-C_{17} alkanes/alkenes (Schirmer et al., 2010). Homologues of PCC7942_orf1593 were explored to expand product profiles - *Arabidopsis thaliana* CER1 and CER2 in *S. cerevisiae* produced ~86 µg per g DCW C_{27}-C_{31} alkanes (Bernard et al., 2012); *A.*

thaliana CER, *Clostridium acetobutylicum* ACR, and 'tesAL109P in *E. coli* produced 327.8 mg/L C_9 and 136.5 mg/L C_{12} alkanes (Choi and Lee, 2013); *Mycobacterium marinum* CAR and a cyanobacterial ADO in *E. coli* BL21(DE3) led to ~2 mg/L C_{11} alkane (Akhtar et al., 2013); *N. punctiforme* ADO in *E. coli* resulted in ~4.2 mg/L C_7 and ~ 1.0 mg/L C_9 alkanes (Sheppard et al., 2016). In addition, C_5 alkane can be produced in *Y. lipolytica* from linoleic acid catalyzed by a soybean lipoxygenase I (Gmlox1) (Blazeck et al., 2013). ADO protein engineering has also been attempted – mutagenesis of the substrate channel of *Synechococcus elongatus* ADO altered the activity and specificity (Bao et al., 2016); a *Procholorococcus marinus* cADO A134F variant showed increased substrate activities toward C_4-C_{12} aldehydes compared to the wild-type (Khara et al., 2013). Other efforts to increase ADO turnover include: 1) applying ferredixin/ferredoxin-NADP+ reductase system with NADPH native to ADO-expressing cyanobacteria, 2) creating ADO proteins fused to alternative electron transfer systems for 'self-sufficient' catalysis, and 3) engineering ADO with a catalase domain to alleviate hydrogen peroxide inhibition that likely arises from poorly balanced electron transfer between the reducing system and ADO (Andre et al., 2013; Herman and Zhang, 2016; Rajakovich et al., 2015). Together, these strategies improved the ADO turnover rate 300-fold above the wild-type. Crystal structures of several ADO enzymes have been reported leading to the discovery of key residues involved in selectivity and activity – suggesting that further protein engineering is possible (Bao et al., 2016; Jia et al., 2017, 2015; Marsh et al., 2014).

Esters. In *E. coli*, methyltransferases from different organisms, including *Mycobacterium marinum* Mmar_3356 and *Drosophila melanogaster* *Dm*JHAMT, were explored to increase FAME production. Expression of Mmar_3356 in *E. coli* produced ~0.13 mg/L C_{10}, ~0.17 mg/L C_{12}, and ~0.073 mg/L C_{14} FAMEs (Nawabi et al., 2011). Overexpression of *Dm*JHAMT in *E. coli* led to 0.56 g/L C_{12}-C_{14} FAMEs (Sherkhanov et al., 2016). The first report of producing biodiesel in *E. coli* demonstrated that AtfA, a WS/DGAT from *Acinetobacter baylyi* strain ADP1 exhibited activity toward C_{12}-C_{20} chain-lengths (Rainer Kalscheuer et al., 2006; Kalscheuer and Steinbüchel, 2003). Homologs of AtfA have been tested by overexpressing in *E. coli* or yeast and exhibited various substrate specificities: *Marinobacter hydrocarbonoclasticus* DSM 8798 WS in *S. cerevisiae* produced 6.3 mg/L C_{12}-C_{18} FAEE (Deng et al., 2015); *Acinetobacter* sp. ADP1 AtfA in *E. coli* expressing 'TesA L109P produced 22.4 mg/L C_{10}, 363.1 mg/L C_{12} and 92.2 mg/L C_{14} FAEE (Choi and Lee, 2013); *A. baylyi* AtfA in *Y. lipolytica* produced ~142.5 mg/L C_{15}-C_{20} FAEE (Xu et al., 2016); *Marinobacter aquaeolei* VT8 Maqu_0168 M405W variant showed a ~3-fold improved selectivity toward ethanol compared to the wild-type (Barney et al., 2015).

4. The state of the field and future directions

Over the past five years, many studies have improved the production of oleochemicals in common industrial microbes. The most significant accomplishments have led to the development of *S. cerevisiae* as an oleochemical producer, expansion of the products produced by *Y. lipolytica*, deeper understanding of the r-BOX pathway, and demonstration of enzyme engineering techniques to alter product profiles. That said, these substantial advancements have failed to lead to high-volume industrial production of oleochemicals in microbes. The remaining barriers include 1.) approaching the theoretical yield of specific oleochemicals other than TAGs, 2.) selectivity towards desired chain lengths, 3.) tolerance to oleochemical products, 4.) wider understanding of regulation modes in potential oleochemical producing hosts, 5.) identifying lower cost feedstocks to feed to cells. Continued development of synthetic biology tools for engineering genomes, analytical methods for identifying cells capable of elevated oleochemical production, systems biology models for predicting cellular phenotypes, and protein design/evolution methods are key to circumventing these barriers. Given the growth in the community studying oleochemical production in microbes, we anticipate that the next five years will bring continued advancement and the development of commercial ventures for high-value oleochemicals (e.g. octanol, dicarboxylic acids, α-olefins). If low-cost feedstocks can be identified, production of highly-demanded, but low cost oleochemicals (e.g. biodiesel) will follow suit.

5. Acknowledgements

This work was funded by the National Science Foundation (CBET-1703504) and the DOE Center for Advanced Bioenergy and Bioproducts Innovation (U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018420). Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the U.S. Department of Energy or the National Science Foundation.

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7. Tables

Table 1. Summary of yields of oleochemical production from carbon sources using microbes.

	Chain-length	**Theo Yield[c] (g/g)**	**Yield (g/g)[a]**	**Titer (g/L)**	**Carbons Source, Medium**	**Hosts**	**Citations**
MK	**C_{11}-C_{17}**	0.32 C_{15}	0.14 g/g fed glucose	3.4 g/L	20 g/L glucose, M9-MOPS minimal medium	*E. coli*	(Goh et al., 2014)
	C_{11}-C_{17}	0.32 C_{15}	0.054 g/g fed glucose	5.4 g/L	Glucose, M9-MOPS minimal medium, fed-batch	*E. coli*	(Goh et al., 2018)
	C_{11}-C_{15}	0.33 C_{13}	10 ug/g DCW	-[d]	20 g/L glucose, YPD medium	*S. cerevisiae*	(Zhu et al., 2017b)
	C_{13}-C_{19}	0.32 C_{15}	0.0074 g/g fed glucose	0.3148 g/L	glucose, YSC medium, fed-batch	*Y. lipolytica*	(Hanko et al., 2018)
FAOH	**C_{6}-C_{10}**	0.34 C_{8}	0.084 g/g consumed glucose	0.42 g/L	20 g/L glucose, minimal medium	*E. coli*	(Dellomonaco et al., 2011)
	C_{4}-C_{16}	0.33 C_{12}	0.2 g/g consumed glucose	1.8 g/L	10 g/L glucose, LB medium	*E. coli*	(Mehrer et al., 2018)
	C_{10}-C_{16}	0.32 C_{14}	0.043 g/g consumed glucose	1.3 g/L	glucose, SC medium, fed-batch	*S. cerevisiae*	(Sheng et al., 2016)
	C_{10}-C_{18}	0.32 C_{14}	0.012 g/g fed glucose	0.1 g/L	20 g/L glucose, YSC medium	*S. cerevisiae*	(Runguphan and Keasling, 2014)
	C_{10}-C_{18}	0.32 C_{14}	0.058 g/g consumed glucose	6.0 g/L	Glucose, YSC medium, fed-batch	*S. cerevisiae*	(d'Espaux et al., 2017)
	C_{22}	-[d]	0.0028 g/g fed glucose	0.0835 g/L	30 g/L glucose, minimal medium	*S. cerevisiae*	(Yu et al., 2017)
	C_{16}-C_{18}	0.32 C_{16}	0.00342 g/g fed glucose	2.15 g/L	60 g/L glucose, YSC medium	*Y. lipolytica*	(Xu et al., 2016)
	C_{16}	0.32 C_{16}	0.004 g/g fed glucose	0.69 g/L	160 g/L glucose, YSC medium	*Y. lipolytica*	(Wang et al., 2016)
FFA	**C_{14}-C_{18}**	0.35 C_{16}	0.28 g/g consumed glucose	~7 g.L	30 g/L glucose, minimal medium	*E. coli*	(Dellomonaco et al., 2011)
	C_{8}	0.42 C_{8}	0.17 g/g consumed glucose	1.7 g/L	4 g/L glycerol, LB medium	*E. coli*	(Hernández Lozada et al., 2018)
	C_{6}-C_{10}	0.42 C_{8}	0.0215 g/g fed glucose	0.43 g/L	20 g/L glycerol, modified MOPS minimal medium	*E. coli*	(Clomburg et al., 2018)
	C_{6}-C_{10}	0.42 C_{8}	0.28 g/g consumed glucose	3.8 g/L	15 g/L glucose, modified MOPS minimal medium	*E. coli*	(Wu et al., 2017a)
	C_{10}	0.39 C_{10}	0.1 g/g fed glycerol	2.1 g/L	20 g/L glycerol, modified MOPS minimal medium	*E. coli*	(Kim and Gonzalez, 2018)
	C_{12}	0.37 C_{12}	0.1 g/g fed glycerol	0.4 g/L	4 g/L glycerol, LB medium	*E. coli*	(Grisewood et al., 2017)
	C_{12}-C_{20}	0.35 C_{16}	0.26 g/g consumed glucose	5.2 g/L	20 g/L glucose, M9 minimal medium	*E. coli*	(Zhang et al., 2012b)
	C_{6}-C_{8}	0.42 C_{8}	0.0232 g/g fed glucose	0.464 g/L	20 g/L glucose, YPD medium	*S. cerevisiae*	(Gajewski et al., 2017)
	C_{6}-C_{12}	0.39 C_{10}	0.00875 g/g fed glucose	0.175 g/L	20 g/L glucose, YPD medium	*S. cerevisiae*	(Zhu et al., 2017b)
	C_{14}	0.36 C_{14}	0.00263 g/g fed glucose	0.052 g/L	20 g/L dextrose, SDC-A medium	*S. cerevisiae*	(Fernandez-Moya et al., 2015)
	C_{16}-C_{18}	0.35 C_{16}	0.02 g/g fed glucose	~0.4 g/L	20 g/L glucose, YSC medium	*S. cerevisiae*	(Runguphan and Keasling, 2014)
	C_{16}-C_{18}	0.35 C_{16}	~0.034 g/g consumed glucose	10.4 g/L	Glucose, minimal medium, fed-batch	*S. cerevisiae*	(Zhou et al., 2016)
	C_{16}-C_{18}	0.35 C_{16}	~0.07 g/g consumed glucose	33.4 g/L	Glucose, minimal medium, fed-batch	*S. cerevisiae*	(Yu et al., 2018)
	C_{12}-C_{18}	0.35 C_{16}	0.022 g/g fed glucose	1.3 g/L	60 g/L glucose, YSC medium	*Y. lipolytica*	(Xu et al., 2016)
	C_{16}-C_{18}	0.35 C_{16}	0.087 g/g fed glucose	10.4 g/L	120 g/L glucose, YNB medium	*Y. lipolytica*	(Ledesma-Amaro et al., 2016)
ALK	**C_{7}**	0.26 C_{7}	0.00035 g/g fed glucose	~0.0042 g/L	12 g/L glucose, LB medium	*E. coli*	(Sheppard et al., 2016)
	C_{7}-C_{9}	0.27 C_{9}	0.0001 g/g fed glucose	~0.0012 g/L	12 g/L glucose, LB medium	*E. coli*	(Sheppard et al., 2016)

	C_9-C_{13}	0.27 C_{11}	n.p.[b]	0.581 g/L	glucose, MR minimal medium, fed-batch	*E. coli*	(Choi and Lee, 2013)
	C_{11}-C_{13}	0.27 C_{11}	0.000951 g/g fed galactose	0.0038 g/L	4 g/L arabinose, LB medium	*E. coli*	(Bao et al., 2016)
	C_{13}-C_{17}	0.28 C_{15}	0.0213 g/g fed glucose	0.426 g/L	20 g/L glucose, M9 modified minimal medium	*E. coli*	(Fatma et al., 2018)
	C_{15}	0.28 C_{15}	0.00233 g/g consumed glucose	0.14 g/L	glucose, M9 minimal medium, fed-batch	*E. coli*	(Q. Liu et al., 2015)
	C_7-C_{13}	0.27 C_{11}	0.00015 g/g fed glucose	0.003 g/L	20 g/L glucose, Delft minimal medium	*S. cerevisiae*	(Zhu et al., 2017a)
	C_{13}-C_{17}	0.28 C_{15}	0.000027 g/g fed glucose	~0.00081 g/L	30 g/L glucose, minimal medium	*S. cerevisiae*	(Zhou et al., 2016)
	C_{11}-C_{19}	0.28 C_{15}	0.000123 g/g fed glucose	0.0037 g/L	30 g/L glucose, YPD+G418 medium	*S. cerevisiae*	(B. Chen et al., 2015)
	C_{13}-C_{17}	0.28 C_{15}	0.000383 g/g fed glucose	0.023 g/L	60 g/L glucose, YSC medium	*Y. lipolytica*	(Xu et al., 2016)
FAEE							
	C_{10}-C_{14}	0.36 C_{12}	0.0159 g/g fed glucose	0.477 g/L	30 g/L glucose, MR minimal medium	*E. coli*	(Choi and Lee, 2013)
	C_{12}-C_{18}	0.35 C_{16}	0.031 g/g fed sugar	0.682 g/L	20 g/L beechwood xylan and 2 g/L glucose, M9 minimal medium	*E. coli*	(Steen et al., 2010)
	C_{12}-C_{18}	0.35 C_{16}	0.075 g/g fed glucose	1.5 g/L	20 g/L glucose, minimal medium	*E. coli*	(Zhang et al., 2012a)
	C_8-C_{10}	0.37 C_{10}	n.p.[b]	n.p.	20 g/L glucose, SCD medium	*S. cerevisiae*	(Lian and Zhao, 2015)
	C_{12}-C_{18}	0.35 C_{14}	0.00025 g/g fed sugar	0.005 g/L	2 g/L glucose, 18 g/L galactose YSC medium	*S. cerevisiae*	(Runguphan and Keasling, 2014)
	C_{16}-C_{20}	0.34 C_{18}	0.00228 g/g fed glucose	0.137 g/L	60 g/L glucose, YSC medium	*Y. lipolytica*	(Xu et al., 2016)
FAME	**C_{12}-C_{14}**	0.29 C_{12}	0.037 g/g fed glycerol	0.56 g/L	15 g/L glycerol, TB medium	*E. coli*	(Sherkhanov et al., 2016)
	C_{16}-C_{18}	0.305 C_{16}	0.27 g/g fed glucose	99 g/L lipid-derived FAME	Glucose, YNB medium, fed-batch	*Y. lipolytica*	(Qiao et al., 2017)

[a] Yield is calculated as either g/g consumed carbon source or g/g fed carbon source, where the former is directly reported in the original papers and the latter is quantified by dividing the reported titer (g/L) over the initial carbon concentration.

[b] Not reported.

[c] Theoretical yield is calculated using a genome-scale metabolic model through fatty acid synthesis pathways under aerobically growth condition.

[d] Not available