Metabolic Engineering 29 (2015) 1–11

Contents lists available at ScienceDirect

Metabolic Engineering

journal homepage: www.elsevier.com/locate/ymben

Minireview

Metabolic engineering strategies for microbial synthesis of oleochemicals

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ARTICLE INFO

Article history:
Received 21 November 2014
Received in revised form
27 January 2015
Accepted 28 January 2015
Available online 7 February 2015

Keywords:
Oleochemical
Fatty acid
Metabolic engineering
Synthetic biology
Biodisesel

ABSTRACT

Microbial synthesis of oleochemicals has advanced significantly in the last decade. Microbes have been engineered to convert renewable substrates to a wide range of molecules that are ordinarily made from plant oils. This approach is attractive because it can reduce a motivation for converting tropical rainforest into farmland while simultaneously enabling access to molecules that are currently expensive to produce from oil crops. In the last decade, enzymes responsible for producing oleochemicals in nature have been identified, strategies to circumvent native regulation have been developed, and high yielding strains have been designed, built, and successfully demonstrated. This review will describe the metabolic pathways that lead to the diverse molecular features found in natural oleochemicals, highlight successful metabolic engineering strategies, and comment on areas where future work could further advance the field.

1. Introduction

Oleochemicals are a class of aliphatic molecules derived from lipids (Biermann et al., 2011). Oleochemicals are used in a wide range of applications including transportation fuels, consumer products (e.g. cosmetics, shampoo, cleaners), and industrial products (e.g. surface coatings, paints, lubricants, bioplastics). The most common oleochemicals are surfactants (e.g. sodium dodecyl sulfate) and biodiesel. Currently, the majority of oleochemicals are made from inexpensive lipid sources such as plant oils and animal fats (Fig. 1). Growing demand for oleochemicals, and in particular biodiesel, has led to an increased production of plant oil crops and raised concern about the sustainability and environmental impact of oil seed production (Fargione et al., 2008). Consequently, interest in identifying alternative oleochemical feedstocks, such as waste oil, meat processing waste, and algal lipids has grown. Alternatively, a wide range of feedstocks, including sugar crops, lignocellulosic biomass, natural gas, and/or carbon dioxide, could be used if microbial biocatalysts were developed via metabolic engineering (Keasling, 2010). Over the last decade, tremendous progress has been made in identifying critical enzymes, increasing understanding of how lipid synthesis is regulated, and demonstrating successful metabolic engineering strategies (Janßen and Steinbüchel, 2014; Klug and Daum, 2014; Lennen and Pfleger, 2013). This review will provide an overview of oleochemical metabolism, highlight some of the successful metabolic engineering approaches, and provide a perspective on remaining challenges in the field.

2. Current and future oleochemical markets

Currently, plant oils are the major feedstock for oleochemical production. As a consequence of growing oleochemical demand, production of plant oils has steadily increased (up 14% from 148.96 Mt in 2010–11 to 169.56 Mt in 2013–14) and is expected to increase further (up 28% by 2023 relative to the 2011–2013 average). Usage of plant oils can be divided into three categories: food, biodiesel and oleochemicals. Of the 157 Mt of plant oil consumed in 2012–13, 77% were used for production of food, 12% were used for production of biodiesel, and the remaining 11% for production of oleochemicals (OECD, 2014; Oilseeds: World Markets and Trade, 2014). Production of vegetable oil is generally considered environmentally unsustainable, due in part to deforestation associated with establishing new palm and soy oil plantations and the long time required to repay the carbon debt associated with the establishing new oil crops (Fargione et al., 2008). Additionally, the use of edible

Abbreviations: Mt, Metric ton; CoA, Coenzyme A; 2-MP-CoA, 2-methyl-propionyl-CoA; 3-MB-CoA, 3-methyl-butyryl-CoA; 2-MB-CoA, 2-methyl-butyrl-CoA; ACCase, Acetyl-CoA carboxylase; FFA, Free fatty acid; FAEE, Fatty acid ethyl ester; FAME, Fatty acid methyl ester; AAce, Acetate acyl ester; MK, Methyl-ketone; Alk, Alkane

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http://dx.doi.org/10.1016/j.ymben.2015.01.009
1096-7176/

vegetable oil for industrial oleochemical production adds to the debate over whether land should be farmed for fuel or food. This problem will continue to grow in the near future since the percentage of global vegetable oil production used for biodiesel is projected to increase from 12% in 2012 to 14% in 2023 (OECD, 2014).

Two potential solutions include the production of lipids in photoautotrophic algae or conversion of plant biomass using engineered microbes. Building upon the early work of the Aquatic Species Program at the National Renewable Energy Laboratory (Sheehan et al., 1998), significant progress has been made using systems biology, synthetic biology, and metabolic engineering to increase the production of algal lipids (Radakovits et al., 2010). Despite these successes, many challenges to algal lipid production remain (Wijffels and Barbosa, 2010). Alternatively, technologies developed for the production of cellulosic ethanol can be utilized to produce biodiesel and oleochemicals with the assistance of novel microbial catalysts. In this approach, microbes are engineered to retask pathways traditionally reserved for production of structural or energy storage molecules for producing specific oleochemical products. The diversity of pathways for producing oleochemical products has been cataloged by many past reviews (Janßen and Steinbüchel, 2014; Lennen and Pfleger, 2013; Peralta-Yahya et al., 2012; Shi et al., 2011; Zhou et al., 2014).

3. Oleochemical metabolism

Oleochemicals are synthesized via enzymatic reactions that use free fatty acids or acyl-thioesters as substrates. Therefore, metabolic engineering strategies for producing oleochemicals by microbial cells begin by redirecting carbon flux from fatty acid metabolism towards a desired product. Fatty acid metabolism is a complex, highly-regulated network of enzymatic reactions that operates on acyl-thioester intermediates (Chan and Vogel, 2010; Janßen and Steinbüchel, 2014; Tehlivets et al., 2007; Zhang et al., 2008). The network uses an iterative reaction pathway to produce a wide-range of acyl-chains from a small set of building blocks. The biosynthetic pathway for any oleochemical can be divided into four processes: chain initiation, chain elongation, chain termination, and chain modification. Each of these processes provides opportunities for customizing a pathway to produce a novel oleochemical. Fig. 2 highlights the options provided by each of these four processes. This review will focus on the two common metabolic engineering hosts, *Escherichia coli* and *Saccharomyces cerevisiae.* The differences between and general advantages of these microbes for producing chemicals have been described elsewhere (Na et al., 2010; Woolston et al., 2013). In terms of oleochemicals, each synthesizes fatty acids from a distinct pathway (bacterial type II FAS and fungal type I FAS, respectively), that provides advantages depending on the desired oleochemical target. These advantages will be highlighted below.

3.1. Initiation

Fatty acid synthesis is primed by short acyl-Coenzyme A (acyl-CoA) thioesters. Incorporation of specific primers determines whether the final acyl-chain will be even or odd and whether the chain will be straight or contain branches. For example, in *E. coli*, even, straight-chain fatty acids are primed by condensation of acetyl-CoA with malonyl-ACP to yield acetoacetyl-ACP, CoA, and CO_2. The reaction is catalyzed by β-ketoacyl-acyl carrier protein synthase III (FabH) (Heath and Rock, 1996). Similarly, odd, straight chain fatty acids are made by *E. coli* when cells are provided with a source of propionyl-CoA, e.g. exogenous propionate or a heterologous propionyl-CoA synthesis pathway (Torella et al., 2013; Tseng and Prather, 2012). Odd chain fatty acids are made because *E. coli* FabH has activity on propionyl-CoA (Heath and Rock, 1996). Homologs of FabH in other bacteria such as *Bacillus subtilis*, have activity on a wider range of substrates including branched acyl-CoA (Choi et al., 2000; Kaneda, 1991). When excess valine is fed to cells, it is converted to 2-methyl-propionyl-CoA (2-MP-CoA). Initiation with 2-MP-CoA leads to the formation of iso-branched, odd chain fatty acids (Choi et al., 2000; Howard et al., 2013). Analogously, leucine and isoleucine can be converted to 3-methyl-butyryl-CoA (3-MB-CoA) and 2-methyl-butyrl-CoA (2-MB-CoA) leading to the

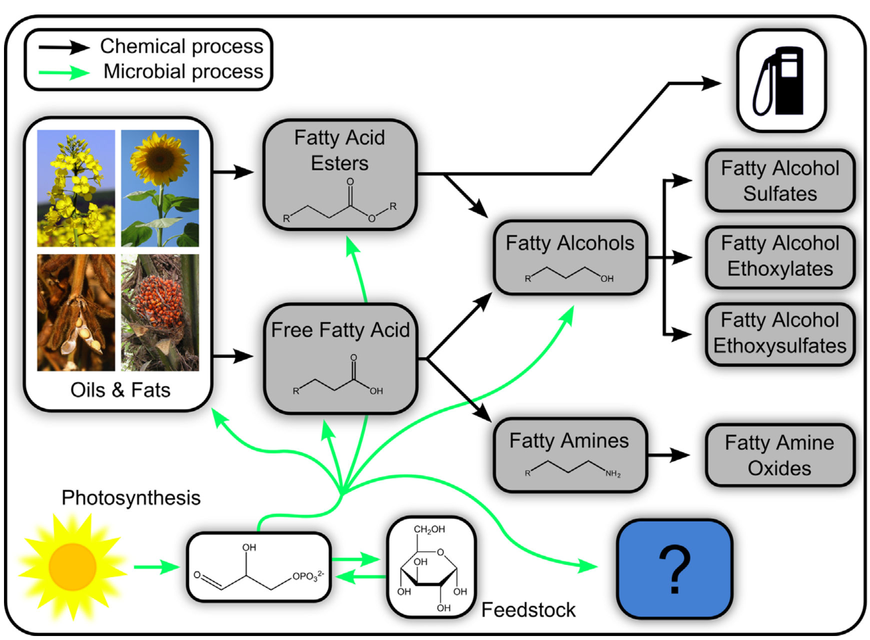

Fig. 1. *Oils and fats are currently the primary feedstock for oleochemical production*. Free fatty acids and fatty acid esters are the raw materials used to synthesize oleochemicals. These intermediates are produced by saponification or transesterification of oils and fats, respectively. Fatty acid esters are used as biodiesel, a renewable transportation fuel with properties comparable to conventional petrodiesel. Fatty acid esters and free fatty acids are converted to fatty alcohols or fatty amines which are intermediates for production of various surfactants such as fatty alcohol sulfates and fatty alcohol ethoxysulfates (anionic) or fatty alcohol ethoxylates and fatty amine oxides (non-ionic). Engineered microbes have the potential to produce many of the intermediates in this scheme from renewable resources. These microbes are either directly photoautotrophic or use plant biomass as a feedstock (such as lignocellulosic sugars).