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Engineering microbial fatty acid metabolism for biofuels and biochemicals

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Traditional oleochemical industry chemically processes animal fats and plant oils to produce detergents, lubricants, biodiesel, plastics, coatings, and other products. Biotechnology offers an alternative process, where the same oleochemicals can be produced from abundant biomass feedstocks using microbial catalysis. This review summarizes the recent advances in the engineering of microbial metabolism for production of fatty acid-derived products. We highlight the efforts in engineering the central carbon metabolism, redox metabolism, controlling the chain length of the products, and obtaining metabolites with different functionalities. The prospects of commercializing microbial oleochemicals are also discussed.

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Introduction

Oleochemicals are a large group of fatty-acid derived compounds with an unprecedented application range: biodiesel, detergents, soaps, personal care products, industrial lubricants, plastic enhancers, bioplastics, emulsifiers, coatings, food and feed additives, and others [1]. Oleochemicals have traditionally been derived from vegetable oils and animal fats via chemical or enzymatic processes [2,3]. However, the limited availability, sustainability, and high cost of feedstocks limit the growth of this sector [4]. The long-term solution for this problem is sought in the expansion of feedstock range to more abundant lignocellulosic biomass [5] and its conversion via chemical and microbial.

Using a microbial chassis in comparison to the traditional conversion of plant oils and animal fats presents a number of advantages. Firstly, feedstock availability is expanded from edible plant oils and animal fats to abundant first-generation and second-generation biomass feedstocks. Secondly, the feedstock-product dependence is eliminated as the desired oleochemicals can be obtained directly using an engineered cell factory from any feedstock, that is, a feedstock can be chosen based on the market price and availability. Finally, complex oleochemicals that cannot be obtained from natural sources because of low abundance can be produced by introducing novel synthetic biochemical pathways into platform chassis.

Microbial chassis must be extensively engineered in order to produce oleochemicals at high titer, rate, and yield for commercial exploitation [6]. The supply of metabolic precursors, acyl-CoAs, and redox co-factor NADPH need to be boosted (Figure 1). The chain length of the products needs to be controlled to obtain the required properties. One must also implement heterologous enzymes that will functionalize fatty acyl-CoAs into final products: hydroxylated and desaturated fatty acids, fatty alcohols, hydrocarbons, waxes, lactones, and others.

This review highlights the recent advances in the engineering of microbial metabolism towards the optimized production of natural and synthetic fatty-acid derived products. The microbial hosts covered in this review include common industrial workhorses: the bacterium *Escherichia coli*, the yeast *Saccharomyces cerevisiae*, and the oleaginous yeast *Yarrowia lipolytica*.

Engineering the central carbon metabolism

A common starting point for engineering microbial hosts is increasing the supply of fatty acid metabolic precursors: acetyl-CoA, malonyl-CoA, and fatty acyl-CoAs. In *E. coli*, it is effective to limit the fermentative pathways towards lactate, acetate, succinate, and ethanol, which consume acetyl-CoA. To circumvent the negative effects of these deletions on the cellular metabolism, these pathways can be first downregulated in the production phase, for example, Wu *et al.* applied CRISPR-based interference for repression of fermentative pathways and achieved 36% increase of the medium-chain fatty acid (MCFA) titer [7] (Table 1).

Table 1

Metabolic engineering strategies for fatty acid-derived metabolites.

Goals and genetic modifications[a]	Achievements	Host	Ref.
Improving acetyl-CoA supply			
Inducible ↓*adhE*, ↓*pta*, ↓*poxB*, ↓*ldhA*, ↓*frdA*)	1.36x C6-C10 FFA titer	Ec	[7]
Acetate recycling (↑*acs*)	3.71x intracellular AcCoA level	Ec	[7]
↑*PCC* ↑MCC	2x FFA	Ec	[17]
↑*ADH2*, ↑*ALD6*, ↑*acs*^{FR}	1.6–1.8x FAEE titer	Sc	[9]
↑*Yl*ACL, ↓*MLS1*, Δ*gpd1*	1.67x FFA titer	Sc	[11]
ACL route (↑*Mmus*ACL ↑*Rt*ME ↑*MDH3'* ↑*CTP1*)	1.18x FFA titer	Sc	[10•]
PDH-bypass (↑*ScPDC1* ↑*EcAldH*)	1.57x lipid titer	Yl	[12••]
Pyruvate-formate lyase route (↑*EcPflA* ↑*EcPflB*)	1.47x lipid titer	Yl	[12••]
Carnitine shuttle (↑*ScCAT2*)	1.75x lipid titer	Yl	[12••]
Non-oxidative PPP (↑*An*PK ↑*BsPta*)	1.62x lipid titer	Yl	[12••]
Increasing acetyl-CoA carboxylase flux			
Minimize acyl-CoA pool (Δ*faa1* Δ*faa4* ↑*Mmus*Tes)	4x ACC transcript level	Sc	[13]
↑*ACB1* ↑*ACC1*^{FR}	1.2–1.3x FAEE titers	Sc	[9]
↑*OLE1* ↑*ACC1* ↑*DGA1*	3x lipid yield, 84.7% TM	Yl	[14•]
Chain-length control			
RBO (↑*RtBktB* ↑*fadB* ↑*Eg*TER ↑*ydiI*) Δ*fadA* Δ*tesB*	1.1 g/L C6–C10 FFA (SF)	Ec	[29]
RBO (↑*RtBktB* ↑*fadB* ↑*Eg*TER ↑*ydiI*) ↑*acs* CRISPRi	3.8 g/L C6–C10 FFA (SF)	Ec	[7]
RBO (↑cyt*FOX3* ↑*Yl*KR ↑*Yl*HTD ↑cyto*ETR1* ↑*EcEutE*) CaBdhB	12 mg/L C6–C10 FFA (SF)	Sc	[30]
FASI replacement (↑*Ec*FAS Δ*fas2* ↑*RcFatB*)	~54 mg/L C14 (SF)	Sc	[27]
MPT swapping with Tes	380 mg/L C14 (SF)	Yl	[12••]
FAS mutagenesis	118 mg/L C6–C8 FFA (SF)	Sc	[23]
Chimeric *Rt*FAS-*Ac*Tes (↑c*Rt*FAS-*Ab*Tes)	1.4 mg/L C6–C8 FFA, 0.3 mg/L C10–C12 FFA (SF)	Sc	[22•]
Product diversification[b]			
Fatty alcohols (*Mmar*CAR *BsSfp* *Ec*AHR *EcFadD*)	2.15 g/L fatty alcohols (FB)	Yl	[12••]
Akanes (↑*Se*FAR ↑*Se*ADO ↑*Se*Fd/FNR)	1.31 g/L alkanes (FB)	Ec	[46•]
Alkenes (↑*Je*OleT ↑*HEM3* Δ*ctt3* Δ*cta* Δ*ccp1*)	3.7 mg/L (FB)	Ec	[47]
FAEEs (ER-localized *AbAtfA*)	136 mg/L (SF)	Yl	[12••]
Linoleic acid (*Fv*d12)	1.3 g/L (SF)	Rt	[51]
GLA (↑*Mal*d6)	71.6 mg/L (SF)	Yl	[35]
EPA (↑heterologous-C16/C18E, d12, d9, d8, d5, d17)	>25% DCW	Yl	[36•,37]
VLC-WE (Δ*elo3* ↑*ELO2* ↑*Maq*FAR ↑*Sci*WS ↑*ACC1*^{FR})	15 mg/L VLC-WE (SF)	Sc	[48]
CBL lipid (cocoa GPAT, LPAT, and DGAT)	Titer not mentioned	Sc	[38]
HFA (Δ*fadD* ↑ACC ↑Tes' ↑*BmCYP102AI*)	58.7 mg/L HFA (SF)	Ec	[40]
Methyl ketones (↑*Rt*FAS-*ShMks2* ↑*ShMks1*)	10 μg/gDCW (SF)	Sc	[22•]
Diacids (RBO, ↑*PpAlkBGT* ↑*AcChnD* ↑*AcChnE*)	0.5 g/L C6–C10 diacids (SF)	Ec	[29]
Docosanol (Δ*elo3* ↑*ELO1,2* ↑*ACC1*^{FR} ↑*AtFAR* ↑*MvFAS*)	83.5 mg/L (SF)	Sc	[24]
Compartmentalization			
Per-*AbAtfA* or ER-*AbAtfA*	15x- (ER) to 19x- (Per) FAEE titer	Yl	[12••]
ER-*Ab*FAR and ER-*Pm*ADO	5.3x alkane titer	Yl	[12••]
Per-*Maq*FAR	2.2x fatty alcohol titer	Yl	[12••]
Per-*Mmar*CAR, Per-*Se*ADO, and Per-*Se*Fd/FNR	1.9x alkanes titer	Sc	[31•]
Redox and cofactor engineering			
Increasing NADPH supply (↑*CaGapC* ↑*McMCE2*)	99 g/L lipid titer (FB, 98% TM)	Yl	[19••]
The use of compatible Fd/FNR for ADO	1.7x alkane titer	Ec	[46•]
*Je*OleT cofactor supply (↑*HEM3* Δ*ctt3* Δ*cta* Δ*ccp*)	1.2x alkene titer	Sc	[47]

The listed examples report the highest titers for corresponding products within the reviewed publications. **Symbols and prefixes —** '↑': overexpression; '↓': downregulation; 'Δ': deletion; cyto: cytosol-localized; '': truncated version. **General abbreviations —** CRISPRi: CRISPR-based interference; SF: shake-flask; FB: fed-batch bioreactor; DCW: dry-cell weight. **Species abbreviations —** *Ab: Acinetobacter baylyi; Ac: Acinetobacter* sp.; *An: Aspergillus nidulans; Bm: Bacillus megaterium; Bs: Bacillus subtilis; Ca: Clostridium acetobutylicum; Ec: E. coli*; *Eg: Euglena gracilis; Fv: Fusarium verticillioides; Je: Jeotgalicoccus* sp.; *Mal: Mortierella alpina; Maq: Marinobacter aquaeolei; Mc: Mucor circinelloides; Mmar: Mycobacterium marinum*; *Mmus: Mus musculus; Mv: Mycobacterium vaccae; Pm: Prochlorococcus marinus*; *Pp: Pseudomonas putida; Rc: Ricinus communis; Rt: R. toruloides; Sci: Simmondsia chinensis; Se: Synechococcus elongatus; Sh: Solanum habrochaites; Yl: Y. lipolytica.* **Gene/Enzyme abbreviations —** ACC: acetyl-CoA carboxylase; ADO: fatty-aldehyde deformylating oxygenase; AHR: aldehyde reductase; CAR: carboxylic acid reductase; C16/C18E: elongase converting palmitate (C16) to stearate (C18); d5, d8, d9, d12, d17: Δ5-desaturase, Δ8-desaturase, Δ9-desaturase, Δ12-desaturase, Δ17-desaturase, respectively; DGAT: diacylglycerol O-acyltransferase; FAR: fatty acyl-CoA- or fatty acyl-ACP reductase; FAS: fatty-acid synthase; Fd/FNR: ferredoxin and ferredoxin/NADP$^+$ reductase; GPAT: glyceryl-3-phosphate acyltransferase; HTD: β-hydroxyacyl-CoA dehydratase; KR: β-ketoacyl-reductase; LPAT: lysophosphatidate acyltransferase; ME: malic enzyme; Sfp: phosphopantetheinyl transferase; TER: trans-β-enoyl-CoA reductase; Tes: thioesterase; WS: wax-ester synthase. **Product abbreviations —** FAEE: fatty-acid ethyl esters; FFA: free fatty acids; HFA: hydroxylated fatty-acids; PK: phosphoketolase; VLC-WE: very-long-chain wax ester.

[a] Unless indicated by a two-letter prefix of the species' name, the genes are native versions.

[b] Except for GLA and EPA, only examples using minimal medium were considered.