AIMS Bioengineering, 3 (4): 468-492.
DOI: 10.3934/bioeng.2016.4.468
Received: 30 September 2016
Accepted: 21 November 2016
Published: 25 November 2016

http://www.aimspress.com/journal/Bioengineering

Review

The use of metabolic engineering to produce fatty acid-derived biofuel and chemicals in *Saccharomyces cerevisiae*: a review

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Abstract: Production of fatty acid-derived biofuels and chemicals have garnered attention in recent years owing to their potential to replace petroleum and plant oil-derived products. Through the metabolic engineering of the fatty acid metabolism pathway, advanced fuels and chemicals such as free fatty acid, triacylglycerol, biodiesel, fatty alcohols, alkanes/alkene, R-3-hydroxybutyric acid, polyhydroxyalkanoates and flavonoids have been produced. The robustness, high tolerance to organic solvent, good reputation in industrial fermentations and excellent availability of genetic tools make the yeast *Saccharomyces cerevisiae* a suitable cell factory for fatty acid-derived biofuels and chemicals production. This review will describe the successful metabolic engineering strategies employed to produce the fatty acid-derived bio-products in *S. cerevisiae*, including the enhancement of precursors and co-factors supply, promotion of the enzyme expression and activity, elimination of competing pathways, and the improvement of strain tolerance.

Keywords: *Saccharomyces cerevisiae*; metabolic engineering; fatty acid metabolism; biofuels and chemicals

1. Introduction

Arising concerns over the energy and environment problems from petroleum industry, have stimulated interest in producing biofuels and chemicals from a renewable source. Metabolic

engineering of microbes which are grown on a sustainable feedstock have been widely used as an alternative to produce fatty acid-derived biofuels and chemicals [1–4]. This includes fatty alcohols, alkanes, alkenes, and fatty esters (biodiesel). There are several advantages in microbial synthesized fatty acid-derived biofuels and chemicals. Firstly, fatty acyl-CoA/ACP and free fatty acids (FFA) are easily converted into many other molecules through biochemical methods [5,6,7]. Secondly, fatty acid-derived biofuels and chemicals are similar to petroleum-based fuels and chemicals, and has been shown to be compatible for use in existing transportation infrastructures. Thirdly, fatty acid molecules are well studied, as they play essential roles in many living organism. In addition, metabolic engineering enables the possibility of combining the microbe's natural fatty acid metabolic pathway with heterologous pathways, and allows diversion of the cell's carbon flux towards the fatty acid pathway so as to increase the fatty acid-derived products production.

S. cerevisiae as a model eukaryotic cell, has been widely explored for use in biochemicals production [8]. Although the number of literature published on fatty acid production using *S. cerevisiae* is far more limited than *E. coli*, *S. cerevisiae* has its own advantages which makes it an appealing host. It is a robust industrial organism that can grow under low pH and various harsh environmental conditions. It has a fully sequenced genome [9], availability of good genetic tools, and well characterized metabolic pathways [10]. Moreover, it has better organic solvent tolerance and well-established industry fermentation techniques. Hence, in recent years, there is increased interest in developing *S. cerevisiae* as a cell factory for fatty acid-derived product production. Besides *S. cerevisiae*, oleaginous yeasts such as *Yarrowia lipolytica* and *Rhodosporidium toruloides* have attracted great attention as their ability to accumulate high levels of lipids. Although several genetic tools have been developed in these oleaginous yeasts [11,12,13], high level gene expression and genetic parts modification tools are still lacking in them, which limit the use of them in biofuel production. But, they are promising hosts, and the study of oleaginous yeasts greatly assist the improvement of the fatty acid accumulation in *S. cerevisiae*, for example ATP dependent citrate lyase (ACL) and malic enzyme from oleaginous yeast have been widely used in biofuel production in *S. cerevisiae* [14,15]. This review will mainly describe the recent advances in *S. cerevisiae*, including heterologous enzyme expression and optimization, competing pathways elimination, precursor and cofactor supply enhancement, and product toxicity avoidance [2,16,17]. Some progress reached in oleaginous yeast will also be briefly summarized.

2. Fatty Acid Metabolism in *S. cerevisiae*

2.1. Fatty acid synthesis and regulation

Fatty acids are usually contained in an esterified form as triglycerides or phospholipids in the cells, or are known as free fatty acids (FFA) when not attached to other molecules [18]. Fatty acids are important compounds in biological systems. They are responsible for various biological functions such as maintaining membrane fluidity, taking part in signaling reactions, modifying protein, storing and supplying energy [19]. A fatty acid is synthesized from acetyl-CoA through the action of the acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) [20]. There are two types of FAS systems. Type I FAS system is used in mammals and yeast, and consists of two subunits. Type II FAS

system is used in most bacteria and mitochondria or chloroplasts of eukaryotic cells, and carries out its catalysis by sets of isolated enzymes.

The mechanism of fatty acid synthesis in *S. cerevisiae* is well studied. Fatty acid can be synthesized both in the cytoplasm and mitochondria. The FAS system in mitochondria belongs to the type II FAS system, while the type I cytosolic FAS system is responsible for synthesizing most functional and storage lipids. The cytosolic acetyl-CoA carboxylase in *S. cerevisiae* is encoded by *ACC1* (*FAS3*) [21]. Acetyl-CoA carboxylase is a large multi-functional enzyme, including the biotin carboxylase domain, the biotin carboxyl carrier protein domain, and the carboxyltransferase domain. The reaction catalyzed by acetyl-CoA carboxylase has been demonstrated as the rate-limiting step in de novo fatty acid synthesis [22]. Similar to *E. coli*, acetyl-CoA carboxylase in *S. cerevisiae* needs ATP and biotin as cofactors for the carboxyl-transfer reaction. The product, malonyl-CoA, is the elongation unit of FAS.

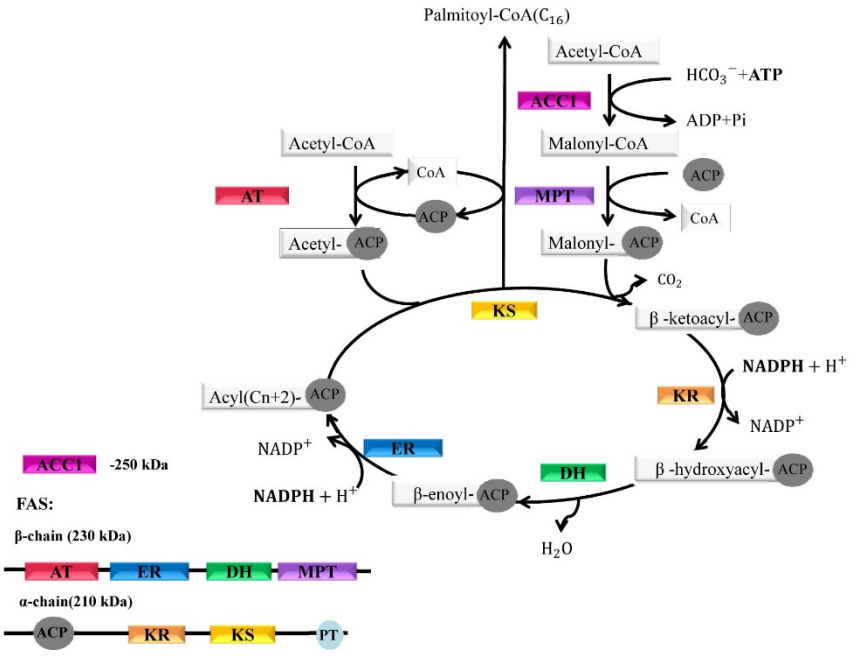

Figure 1. Fatty acid synthesis and elongation in yeast. AT: acetyl-transferase, ER: enoyl reductase, DH: dehydratase, MPT: malonyl-palmitoyl transferase, KS: ketoacyl synthase, KR: ketoacyl reductase, ACP: acyl carrier protein, PT: phosphopantetheine transferase.

The FAS [23–27] in yeast consists of Fas1 (β subunit) and Fas2 (α subunit), and is organized as a barrel-shaped α6β6 complex of 2.5MDa. The α-subunit contains the catalytic centers: phosphopantetheine transferase (PT), ACP, ketoacyl synthase (KS), ketoacyl reductase (KR) and part of the malonyl-palmitoyl transferase (MPT) domain. The β-subunit contains acetyl-transferase (AT), enoyl reductase (ER), dehydratase (DH) and the major part of the MPT domain (Figure 1). AT initiates the fatty acid chain synthesis through loading the acetyl primer from coenzyme A (CoA) to ACP, MPT loads malonyl elongation substrate from malonyl-CoA to ACP. Acetyl and malonyl substrates are condensed to acetoacetyl-ACP by the KS domain. In the subsequent reaction steps, the

β-carbon groups are processed by KR, DH, and ER, which results in fully saturated acyl-ACP extended by two carbon atoms (acyl(n + 2)-ACP). Acyl-ACP can serve directly as a primer for the next condensation reaction. The chain elongation step stops until C16- or C18-acyl-residues are back-transferred to CoA by MPT, yielding long-chain acyl-CoAs [19,25,28]. This is different from bacteria, in which acyl-ACPs was the direct product of type II FASs, acyl-ACPs can be hydrolyzed by a thioesterase (TE) to release FFA, or catalyzed directly to lipids [29]; or mammalian cells in which chain-termination step was catalyzed by a thioesterase domain to release FFA from FAS [30]. Very long chain fatty acids (VLCFA, C20-C26 carbon atoms) are synthesized by C16 or C18 saturated acyl-CoAs and malonyl-CoA in the endoplasmic reticulum. Enzymes Elo1, Elo2, and Elo3 catalyze the condensation or decarboxylation of acyl-CoA with malonyl-CoA [31,32].

The regulation of fatty acid synthesis in yeast is also complicated and occurs at multiple levels, such as gene expression level, protein level, and growth condition regulation. At the gene expression level, genes *ACC1*, *FAS1*, and *FAS2* are all subjected to coordinated control by lipid precursors inositol and choline, and the positive and negative transcription factors Ino2/Ino4 and Opi1, respectively [19]. The negative regulator Opi1p represses UAS_{INO}-containing genes (UAS_{INO} is a cis-acting inositol-sensitive upstream activating sequence), such as the highly regulated gene *INO1*, by inhibiting Ino2p and Ino4p transcription factors [33,34]. Deletion of gene *OPI1* can de-repress the transcriptional regulation of *INO1*, which codes for the rate-limiting enzyme in inositol synthesis, thus leads to irrepressible overproduction of inositol. Because the genes *ACC1* [35], *FAS1*, and *FAS2* [36] also contain UAS_{INO} elements in their promoters, they are regulated by inositol through the same mechanism. Inositol supplementation results in the repression of *ACC1*, *FAS1*, and *FAS2*, and is only less repressed than *INO1* [37,38]. Expression of *ACC1* and *FAS1* are dependent on histone acetylation, and regulated by the SAGA and TFIID complexes. *ACC1* show cell cycle-dependent expression; the peak level appears at G1 phase. *FAS1* and *FAS2* reach the highest level of expression during the M/G1 transition [39], which indicates that there is coordination between the expressions of these genes. Besides, the transport and stability of mRNA might have significant effect on expression efficiency; still, specific information of mRNA stability of these genes is not well studied. There are several potential binding sites for general transcription factors, like Gcr1, Abf1, Grf1 (Rap1), and Reb1, in the promoter regions of the genes *FAS1*, *FAS2*, and *ACC1*. Removal of potential binding sites from the *FAS1* promoter led to a 40% reduction of expression of a reporter gene. Removal of Gcr1-binding site from the *FAS2* promoter resulted in a 90% reduction in expression. Likewise, deletion of Abf1, Reb1, and two Grf1 binding sites sequences from the *ACC1* promoter reduced expression of *ACC1* gene by 54% [40].

On the protein level, Snf1 kinase in yeast phosphorylates and inactivates ACC1 at position serine 1157. Acyl-CoAs, including malonyl-CoA and long chain acyl-CoAs, also inhibit the activity of ACC1, FAS1, and FAS2 [41]. For FAS holoenzyme, the amount is dependent on the level of FAS1. Over-expression of *FAS1* leads to concomitant expression of *FAS2* and substantially enhances FAS activity; on the other hand, *FAS1* expression cannot be regulated by *FAS2* gene dosage. A downstream repression site (DRS) responsible for *FAS1*-dependent expression of *FAS2* was identified within the coding sequence of *FAS2* [42]. Moreover, active FAS complex requires covalent modification of the ACP domain in the α subunit (Fas2) by 4'-phosphopantetheine, which is catalyzed by an intrinsic phosphopantetheine: protein transferase (PPTase) activity in the C-terminal

end of the α subunit [43].

Moreover, different growth conditions affect fatty acid synthesis; for example, the transcription of *ACC1*, *FAS1*, and *FAS2* are all decreased when there are fatty acids in the medium. A study has demonstrated that this is due to the long chain acyl-CoA feedback inhibition, which requires acyl-CoA synthase activity [36,44]. Yeast can rapidly regulate ACC1 activity to adjust to different growth conditions, such as glucose limitation and salt stress. These changes are sensed and transduced to ACC1 by the Snf1 kinase [45]. Furthermore, a study has shown that changing carbon source from ethanol to galactose may increase the level of FAS1 protein [46].

Monounsaturated fatty acids in *S. cerevisiae* are formed by the Δ-9 fatty acid desaturase (OLE1) in the endoplasmic reticulum membrane. Although it is a single enzyme system, the expression of gene *OLE1* is highly regulated and responds to a number of environmental and nutritional stimuli; such as, carbon source, nutrient fatty acids, metal ions, and oxygen levels [47]. Because the double bond formation reaction catalyzed by OLE1 requires oxygen and reducing equivalents from NADH, OLE1 activity was observed to increase by several folds when yeast cells were shifted from fermentative to respiratory growth on glucose. Because mitochondria proliferate in respiratory growth phase, which maybe increase the demand of UFA precursors for membrane lipids synthesis [48]. Another study showed that *OLE1* mRNA levels increased 6-fold when yeast cells were cultured on non-fermentable carbon sources (glycerol or lactate) as compared with glucose, which is because mitochondrial genes are maximally expressed under the obligate respiratory conditions [47]. The content of UFA also exhibits an increase when *S. cerevisiae* adapts to low temperatures [49]; this is mediated by the mitochondria membrane proteins Spt23p and Mga2p. When UFA is added to the culture medium, *OLE1* mRNA levels decrease rapidly due to the transcription repression [50] and mRNA stability control [51]. Moreover, *OLE1* can be regulated by oxygen, certain metals, and irons [52,53].

A concept which need to be taken note of is that there are several sources of fatty acids in the cell, such as de novo synthesis, exogenous fatty acid uptake, protein turnover, and lipid turnover. De novo fatty acid synthesis provides precursors for fatty acid elongation, such as malonyl-CoA and the subsequent longer chain acyl-CoA (C14-C16). The fatty acyl-CoAs are then assembled to form triacylglycerols (TAG) and steryl esters (SE), which belongs to the lipid storage pathway. The neutral lipids which are stored inside the lipid body serve as energy reserves. In the presence of external sources of fatty acids, yeast cells uptake these substrates, and carry out subsequent modification for storage and accumulation. This involves incorporation and transportation of the extracellular fatty acids in the form of CoA-thioesters [54], which is then catalyzed by fatty acyl-CoA synthetases [55].

2.2. Fatty acid degradation and regulation

Microorganisms are able to use fatty acids as carbon sources because they can take up and degrade fatty acids from the environment. In *S. cerevisiae*, fatty acid degradation occurs only in peroxisomes due to the metabolic compartmentalization [56,57]; the breakdown route is showed in Figure 2. Fatty acids are converted to acyl-CoAs and shortened into acetyl-CoA by two carbons each cycle. Final product acetyl-CoA can be used as a building block in anabolic reactions, or catabolized in the TCA cycle. The process requires an array of β-oxidation enzymes including Pxa1p/Pxa2p,

Pox1p/Fox1p, Mfe2p/Fox2p and Pot1p/Fox3p, and need various auxiliary enzymes to supply the proper substrates to the machinery.

Before the degradation steps, fatty acid released from triglycerides or imported from the environment are activated by fatty acid acyl-CoA synthetases [55]. There are six fatty acid acyl-CoA synthetases in *S. cerevisiae*, Faa1p-Faa4p, Fat1p and Fat2p. Faa1p and Faa4p are the main enzymes in cytoplasm. They are needed for activating long chain fatty acids (LCFAs) before they are transported into peroxisome by Pxa1p-Pxa2p. Faa2p and Fat2p are located in peroxisome. It is suggested that Faa2p is involved in activating medium chain fatty acids (MCFAs). Fat1p activates VLCFA (>C20) before oxidation. There is no clear substrates preference for Faa3p. Besides the function of activating fatty acids, acyl-CoA synthetases also are needed in importing fatty acids from environment.

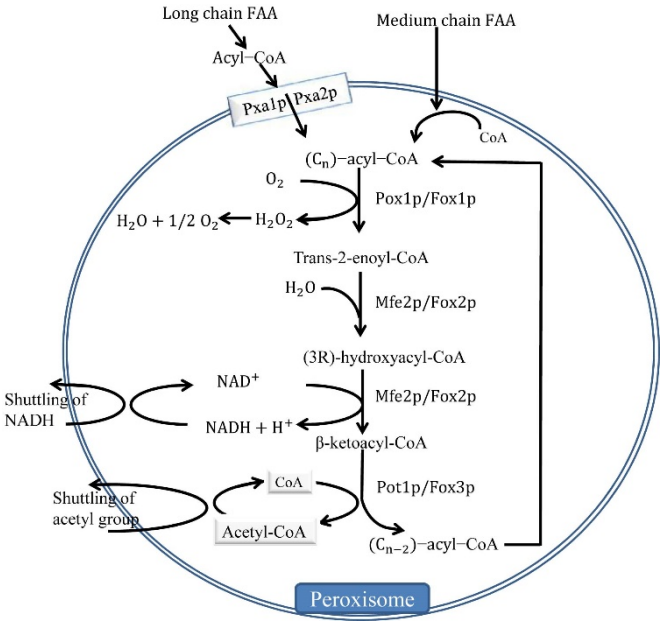

Figure 2. Schematic representation of fatty acid β-oxidation in yeast peroxisomes. This figure is modified based on figure 1 in [56]. Protein Pxa1p-Pxa2p embedded in peroxisomal membrane translocate activated fatty acids into peroxisomes for degradations. The β-oxidation reactions are catalyzed by enzymes Pox1p/Fox1p, Mfe2p/Fox2p and Pot1p/Fox3p.

After LCFAs are activated to their CoA derivatives in the cytosol, they are transported into peroxisome by Pxa1p and Pxa2p transporter; while MCFAs are activated to medium chain acyl-CoA inside peroxisomes [58]. The first step, which is the rate-limiting step of β-oxidation, is catalyzed by acyl-CoA oxidase. *POX1* gene in *S. cerevisiae* encodes the only acyl-CoA oxidase, which can oxidize acyl-CoAs of various chain lengths. Disruption of the *POX1* gene makes yeast cells unable to survive on mediums with oleic acid as a sole carbon source [59]. While in *Y. lipolytica*, there are six acyl-CoA oxidases, each one has different chain length specificity [60,61]. It has been demonstrated that modification of *POX* genotype can affect β-oxidation and further affect lipid accumulation in *Y.*

lipolytica [62]. In the second step, Enoyl-CoA hydratases Mfe2p/Fox2p transform trans-2-enoyl-CoA into β-ketoacyl-CoA via (3R)-hydroxy intermediates. *S. cerevisiae* Mfe2p/Fox2p contain two dehydrogenase domains, domain A is most active with long chain and medium chain substrates, on the contrary, domain B prefers short chain substrates [56]. In the last step of β-oxidation, 3-ketoacyl-CoA thiolase Pot1p/Fox3p cleaves ketoacyl-CoA into acetyl-CoA and a C_2-shortened acyl-CoA, the latter is substrate for Pox1p/Fox1p. Acetyl-CoA is exported into mitochondria for further oxidation in TCA cycle or towards biosynthesis via the glyoxylate cycle [56].

The transcription of genes encoding enzymes in fatty acid β-oxidation can be induced dramatically by the fatty acids in the culture medium. The response of yeast cells is mediated by the Pip2p-Oaf1p transcription factors, which bind to ORE element in the promoters of these genes. It also causes the proliferation of peroxisome [56].

3. Fatty Acid Derived Biofuels and Chemicals Production

To show the potential of *S. cerevisiae* as a biofuel production platform, biofuels that could be produced in *S. cerevisiae* are summarized in Figure 3. After fatty acid profile and production were modified, extra enzymes were integrated in the metabolic pathways to do *in vivo* transformation to produce biofuels and chemicals, such as biodiesel, fatty alcohols, alkanes/alkenes and terminal alkene.

3.1. Fatty acid production

Studies about the manipulation of fatty acid accumulation have been carried out for several decades. There are many reports about increasing the fatty acid production and FFA chain length profile in *E. coli*, by methods such as overexpression of fatty acid synthesis enzymes and different thioesterases, inactivation of β-oxidation pathway and optimization of fermentation condition [63,64,65].

The differences between *S. cerevisiae* and *E. coli* are that: fatty acyl-ACPs are synthesized by a dissociated type II fatty acid synthetase (FAS) system in *E. coli* [66], while *S. cerevisiae* uses type I FAS system and only fatty acyl-CoAs can be released from the FAS complex [19]. Several of type II FAS enzymes are feedback inhibited by long-chain fatty acyl-ACPs [29], while type I fatty acid synthesis is feedback inhibited by long chain acyl-CoAs [44]. It has been shown that overexpression of acyl-ACP thioesterases in *E. coli* can relieve the feedback inhibition, modify the fatty acid chain length profile, and lead to an overproduction of FFA [66]. Similarly, expression of cytosol acyl-CoA thioesterase in *S. cerevisiae* can alleviate the feedback inhibition of long chain acyl-CoAs in a similar fashion as in *E. coli* [67].

The storage forms of fatty acids in *S. cerevisiae* includes TAG, FFA and fatty acyl-CoA. All of them can be utilized as precursors in biofuels and chemicals biosynthesis, especially FFA-derived pathways for the production of high value chemicals have got great attention recently, since FFA can be accumulated to more than 200-fold than fatty acyl-CoA [15]. Besides, producing secreted FFA or its derived product can skip the energy intensive separation steps [66,79]. There has been great progress in engineering *S. cerevisiae* to accumulate fatty acid in recent years. For example, short chain fatty acids were successfully produced in *S. cerevisiae* by using the heterologous Homo

sapiens type I fatty acid synthase (hFAS) with the heterologous short chain thioesterases from *Cuphea palustris* (CpFatB1) or *Rattus orvegicus* (TEII) [80]. About FFA production, Scharnewski et al [81] found that the yeast strain could secrete 220 μmol/L of FFA by double deletion of two main acyl-CoA synthases, FAA1 and FAA4. Besides *FAA1* and *FAA4* disruption, overexpression of *E. coli* acyl-ACP thioesterase in *S. cerevisiae* yielded 207 mg/L FFA, and combined with overexpression of the fatty acid synthase (FAS) and acetyl-CoA carboxylase, the production of FFA increased up to 400 mg/L FFA; Further overexpression of diacylglycerol AT (*DGA1*) led to more than 17% increase of

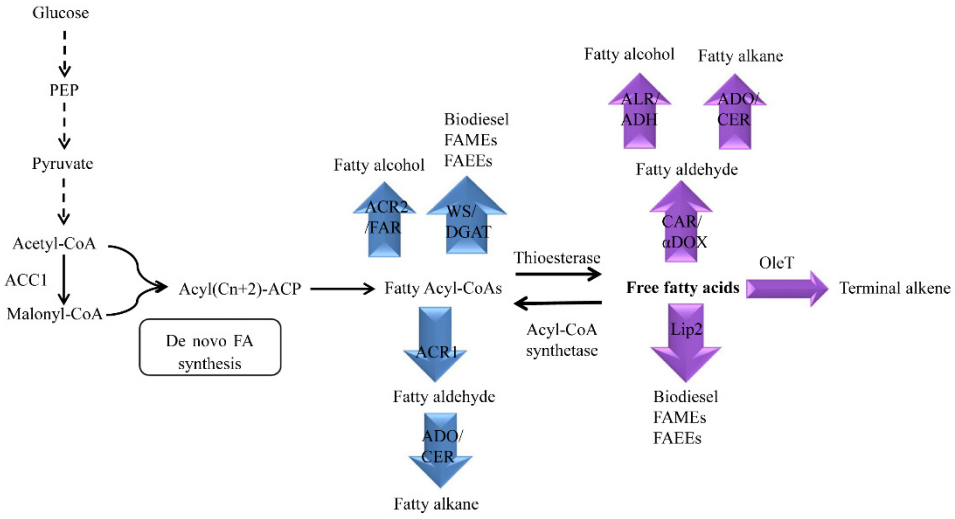

Figure 3. Production of fatty acid-derived biofuels through fatty acid metabolic pathway in *S. cerevisiae.* Dashed lines represent multiple reaction steps. PEP, phosphoenolpyruvate. ACC1: Acetyl-CoA carboxylase; ACP: acyl carrier protein; ACR: fatty acyl-CoA reductase (ACR1 [68] and ACR2 [69]); FAR: fatty acyl-CoA reductases [70]; CAR: carboxylic acid reductase [15,71]; α-dioxygenase (αDOX) [72]; ALR/ADH: alcohol dehydrogenases/aldehyde reductase [15]; ADO: aldehyde deformylating oxygenase [73,74]; CER: aldehyde decarbonylase [75]; Lip2: lipase [76]; WS/DGAT, wax ester synthase/acyl-coenzyme A: diacylgylcerol acyltransferase [77]; OleT: fatty acid decarboxylases [78]. The fatty acid biosynthetic machinery could be utilized to produce these valuable biofuels: alcohols could be produced by reduction of fatty acyl-CoA or FFA to fatty alcohol, whereas alkanes could be produced by reduction of fatty acyl-CoA or FFA to the aldehyde followed by decarbonylation, and biodiesel could be produced by converting fatty acids to esters via esterification with small alcohols by ester synthase.

triacylglycerol [82]. In another study, it was demonstrated that the disruption of the β-oxidation pathway and the acyl-CoA synthetases, overexpression of different thioesterases and enhancement of the supply of acetyl-CoA resulted in the production of more than 120 mg/L FFA [83]. Similarly, the accumulation of FFA up to ~500 mg/L was successfully achieved by double deletion of acyl-CoA synthases genes *FAA1* and *FAA4*, and cytosolic expression of truncated version of acyl-CoA thioesterase Acot5 (Acot5s) from *Mus musculus* [67]. In a recent study, it was found that disruption

of the acyl-CoA synthetase genes *FAA1*, *FAA4* and *FAT1* yielded 490 mg/L FFA, combined with disruption of β-oxidation pathway and co-expression of lipid body forming enzyme *DGA1* and lipase *TGL3*, 2.2 g/L FFA was produced [84].

Using transposon mutagenesis analysis, fatty acid transporters disruptant *Δsnf2* was found to have significant increase in both FFA (4.13 fold) and triacylglycerol (69%) as compared to wild type [85]. In addition, overexpression of acyl-CoA synthase *FAA3* and diacylglycerol acyltransferase *DGA1* in the *Δsnf2* disruptant increased the total lipid approximate 30% [86]. Furthermore, through overexpression of the active diacylglycerol acyltransferase Dga1p lacking the N-terminal 29 amino acids (Dga1ΔNp) in a *Δdga1* mutant, *S. cerevisiae* was transformed into an oleaginous yeast with fatty acids production up to 45% [87].

To date, the highest reported FFA titer produced in engineered *S. cerevisiae* is 10.4 g/L. The modification involved deletion of two of the main fatty acyl-CoA synthetase *FAA1* and *FAA4* to interrupt the reactivation of FFA; deletion of fatty acyl-CoA oxidase *POX1* to prevent fatty acid degradation; expression of a truncated *E. coli* thioesterase *'tesA* to increase FFA production from fatty acyl-CoA; expression of ATP: citrate lyase (*MmACL*) from *Mus musculus*, malic enzyme (*RtME*) from *R. toruloides*, endogenous malate dehydrogenase with removed peroxisomal signal (*'Mdh3*) and citrate transporter *Ctp1* to increase the supply of the precursor cytosolic acetyl-CoA; expression of a *R.toruloides* FAS (*RtFAS)* to enhanced fatty acid synthesis; and replacement of the native promoter of *ACC1* with *TEF1* promoter to moderately enhance the expression of the wild-type *ACC1*. All the modifications gave a FFA production of 1.0 g/L in shake flask cultivation. Through glucose limited fed-batch cultivation of the resulting engineered strain, a titer of 10.4 g/L FFA was achieved [15].

Compared to *S. cerevisiae,* oleaginous yeast can produce much higher level of lipids. With the development of molecular biology tools in oleaginous yeasts, great progress have been reached recently. For example, overexpression of delta-9 stearoyl-CoA desaturase (SCD), acetyl-CoA carboxylase (ACC1) and diacylglyceride acyl-transferase (DGA1) all together in *Y. lipolytica* enhanced cell growth and lipid overproduction simultaneously, and produced 55 g/L lipid under fed-batch fermentation condition [88]. The highest reported lipid titer produced in *Y. lipolytica* is 85 g/L in fed-batch glucose fermentation. The genetic modification involved heterologous overexpression of a DGA1 enzyme from *R. toruloides*, a DGA2 enzyme from *Claviceps purpurea*, and deletion of the native TGL3 lipase regulator [89]. Moreover, MCFAs were produced in *Y. lipolytica* by expression of different acyl-acyl carrier protein (ACP) thioesterases with medium-chain acyl-ACP substrate preference [90]. The highest reported lipid titer produced in another oleaginous yeast *R. toruloides* is 89.4 g/L during fed-batch growth on glucose [91]. The genetic modification included simultaneous overexpression of acetyl-CoA carboxylase (ACC1), diacylglycerol acyltransferase (DGA1) and stearoyl-CoA desaturase (SCD) in *R. toruloides* strain IFO0880. All the progress show the great potential of oleaginous yeast in other lipid-derived compounds production.

3.2. Biodiesel production

Biodiesel is a mixture of fatty acid short-chain alkyl esters, such as fatty acid methyl esters (FAMEs), fatty acid ethyl esters (FAEEs) and fatty acid esters with branched chain alcohol. They are

usually produced by transesterification of different fatty acid carbon chain lengths triacylglycerols or FFA with short chain alcohols [92]. They are potential replacements for diesel fuel because of their low host toxicity and high energy density. Compare to immobilized lipase and chemically catalyzed processes, fatty acids-derived biosynthesis has become a rapidly developing research area for biodiesel production. The main enzymes used in biodiesel biosynthesis are lipase, wax-ester synthase and bacterial fatty acid methyltransferase [93].

Lipases can effectively perform transesterification with TAGs and esterification with FFA at the same time. Most genetic engineering studies of lipases are to produce lipases (includes in intracellular, secreted or surface-displayed forms) by whole cells for whole-cell lipase-mediated in vitro biotransformation. In a recent study, by expressing lipase 2 from *Candida sp.* 99–125 in *S. cerevisiae*, transesterification of triacylglycerol or FFA is accomplished *in vivo*. When combined with an additional 4% ethanol in the culture broth, the yield of FAEEs in yeast reached 11.4 mg/g dry cell weight [76].

Recent studies explore the use of wax-ester synthase with fatty acyl-CoA: alcohol acyltransferase activity to produce FAEEs from fatty acyl-CoA and ethanol in *S. cerevisiae.* One yeast strain expressing wax-ester synthase from *M. hydrocarbonoclasticus* DSM 8798 could produce 6.3 mg/L FAEEs [94]. The further elimination of non-essential fatty acid utilization pathways including β-oxidation and neutral lipid formation pathway, improved FAEEs production to 17.2 mg/L [95]. To overcome the metabolic burden on the host cell and instability of plasmid-based overexpression method, the wax-ester synthase was integrated into the yeast chromosomal, and the corresponding FAEEs production increased up to 34 mg/L [96]. To further improve FAEEs production, the supply of precursor fatty acyl-CoA and cofactor NADPH were enhanced by overexpression of endogenous acyl-CoA binding protein (*ACB1*) and a bacterial NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (*gapN*), another 40% increase of FAEEs production levels was achieved [96]. In another study, a wax-ester synthase from *Acinetobacter calcoacericus* ADP1 (*atfA*) with a broad alcohol substrates preference was heterologously expressed in *S. cerevisiae* WRY1strain, in which all the promoters of fatty acid biosynthesis genes were replaced by the strong constitutive promoter *TEF1*. The engineered strain could produce 4.6 mg/L FAEEs directly from simple sugars. The production levels was further improved to 4.9 and 5.4 mg/L after deleting *PXA2* and *POX1* respectively [82]. Two other wax ester synthase genes (*ws2* and *Maqu_0168*) with different alcohol preferences were expressed in *S. cerevisiae* to produce biodiesel mixture, including fatty acid ethyl, isobutyl, isoamyl and active amyl esters. To further improve the production, gene *Rpd3* and *Opi1* were deleted to increase fatty acyl-CoAs concentration. In addition, isobutanol pathway enzymes (Ilv2, Ilv5, Ilv3, Aro10 and Adh7) were expressed in the mitochondria to enhance the supply of alcohol precursor. Further combine with high-cell-density fermentation, over 230 mg/L fatty acid short- and branched-chain alkyl esters biodiesel were produced [92].

A recent study demonstrated that, in order to bypass the endogenous fatty acid regulation, and supply additional fatty acids to FAEEs synthesis, a Type-I fatty acid synthase (FAS) from *Brevibacterium ammoniagenes* was expressed in yeast. Combined with fatty acid acyl-CoA ligase (*faa1*) and the wax ester synthase (*ws2*), the FAEEs production in the engineered strain increased 6.3-fold as compared to the strain without the type-I FAS [77].

In addition, *S. cerevisiae* also can use other carbon source such as glycerol to produce FAEEs.

For example, by expression of an acyltransferase ADP1 from *Acinetobacter baylyi* and glycerol utilizing genes, the engineered *S. cerevisiae* strain accumulated FAEEs using exogenous fatty acids and endogenous ethanol produced from glycerol. Approximately 0.52 g/L FAEEs was produced with around nearly 17 g/L of glycerol consumed [97]. Besides metabolic modification, culture conditions optimization was also adopted to improve the production of FAEEs. A 40% improvement was reported in nitrogen limitation modification [98].

3.3. Fatty alcohol production

Fatty alcohols are worth a total of three billion dollars in the global market each year. In addition to fuels, fatty alcohols can also be used in various type of products, including lubricants, surfactants, solvents, pharmaceuticals and cosmetics. For sustainable microbial production of fatty alcohols, *S. cerevisiae* seems to be a more efficient host as compared to *E. coli*, as it involves only one or two step catalytic reaction from fatty acyl-CoAs or FFA [99].

There has been great progress in improve fatty alcohol production level through carbon flux control and fatty acid synthesis regulation. By expressing fatty acyl-CoA reductase (TaFAR) from *Tyto alba* in a *Δdga1* strain, the accumulated cellular fatty acyl-CoAs saved from the fatty acyl-CoAs dependent TAGs synthesis was redirected toward fatty alcohol synthesis, 26 mg/L intracellular and 4.3 mg/L extracellular fatty alcohol were achieved. Through optimizing carbon and nitrogen concentration in the culture medium, the fatty alcohols yield in the engineered strain was further increased to 84 mg/L in intracellular cells and 14 mg/L secreted in medium [70]. Overexpression of the mouse fatty acyl-CoA reductase (mFAR1) in a *S. cerevisiae* WRY1 strain, in which all the promoters of fatty acid biosynthesis genes were replaced by the strong constitutive promoter *TEF1*, 93.4 mg/L fatty alcohol was produced from hexose sugars. Further overexpression of the malic enzyme from *Mortierella alpine* increased the fatty alcohol production levels to 98.0 mg/L [82].

In another effort to engineer *S. cerevisiae* for 1-hexadecanol production, a maximum of 45 mg/L fatty alcohols were produced when TaFAR from *Tyto alba* was expressed in the BY4741 strain. When *ACC1* under the strong constitutive promoter *PGK1* was overexpressed, 1-hexadecanol production was enhanced by 56%. When RPD3, the negative regulators of phospholipid metabolism, was further deleted, 1-hexadecanol production was enhanced another 98%. Furthermore, an ATP-dependent citrate lyase was expressed to increase the cytosolic acetyl-CoA, the production of 1-hexadecanol was increased an additional 136% to 330 mg/L. Finally, over 1.1 g/L 1-hexadecanol was produced in glucose minimal medium by fed-batch fermentation using resting cells [14].

Besides even chain length fatty alcohols, odd chain-length fatty alcohols were produced in engineered *S. cerevisiae* by using cytosolic thioesterase, rice α-dioxygenase (αDOX) and endogenous aldehyde reductases. First, a cytosolic thioeseterase was expressed in a *FAA1* and *FAA4* double deletion strain to accumulate more FFA. Next, αDOX was over-expressed to convert even chain FAAs to odd chain fatty aldehydes by oxidative decarboxylation. Then, endogenous aldehyde reductases produce fatty alcohols spontaneously at a production level about 20 mg/L [72]. In addition to αDOX, carboxylic acid reductase (CAR) was showed to have high efficiency to convert FFA into fatty aldehyde. Through expressing a CAR from *Mycobacterium marinum* (MmCAR) in a FFA producing yeast strain *Δfaa1Δfaa4Δpox1*, the endogenous alcohol dehydrogenases convert the

fatty aldehyde intermediates into fatty alcohols, a titer of 23.2 mg/L fatty alcohols were produced. Further deleted *HFD1* to prevent the transformation of aldehyde to FFA, the fatty alcohol production increased to 61.2 mg/L. Combing with co-expression of bi-functional fatty acyl-CoA reductase *FaCoAR* from *Marinobacter aquaeolei* VT8 and alcohol dehydrogenases *ADH5*, the fatty alcohol production was further increased. Furthermore, additional deletion of *ADH6* improved fatty alcohol production to 89.5 mg/L. Together with genome-integrated an additional copy of MmCAR, 28% more fatty alcohols was produced. Glucose limited fed-batch cultivation further significantly improved the production to 1.5 g/L, which is the highest reported fatty alcohols production titer in *S. cerevisiae* [15].

In a recent pathway compartmentalization strategy, medium chain fatty alcohols (decanol and 1-dodecanol) were produced in peroxisome when the fatty acyl-CoA reductase TaFAR was targeted expressed in the peroxisome of *S. cerevisiae.* However, because palmitoyl-CoA is the most abundant fatty acyl-CoA in peroxisome, 1-hexadecanol was still the main fatty alcohols. Next, fatty alcohol production was further improved 1.4 fold by over-expression of genes encoding peroxisomal membrane protein PEX7 and acetyl-CoA carboxylase. Further medium optimization improved the fatty alcohols production at 1.3 g/L in fed-batch fermentation using glucose as carbon source [100].

Besides hexose sugars fermentation, non-edible sugars such as xylose can also be converted to fatty alcohols. For example, 0.4 g/L 1-hexadecanol was produced by expressing a fungal xylose utilization pathway (consisting of xylose reductase XR, xylitol dehydrogenase XDH, and xylulose kinase XKS) in a fatty alcohol producing strain XF3 (expressing TaFAR, ACC1 and ACL under *ΔRPD3* background). Next, the expression levels of XR, XDH and XKS were optimized by combinatorial promoter engineering, the production of 1-hexadecanol was increased by 171%. Furthermore, 1.2 g/L 1-hexadecanol was produced by fed-batch fermentation, which is the highest reported xylose-based 1-hexadecanol production level [101].

Moreover, there has been some promising progress achieved in the oleaginous yeast. In a latest study of *Y. lipolytica*, over 500 mg/L of 1-decanol was produced by heterologous expression of a fatty acyl-CoA reductase from *Arabidopsis thaliana* and deletion of the major peroxisome assembly factor Pex10 [102]. In a another study, through the heterologous expression of an acyl-ACP reductase from *Marinobacter aquaeolei* VT8, *R. toruloides* was engineered to produce over 8 g/L of C16–C18 fatty alcohols in fed-batch condition using sucrose as carbon source [103].

3.4. Alkane/alkene production

As the predominant constituents of gasoline, diesel, and jet fuels, alkane and alkene biosynthesis have got great progresses recently. Since 2010, acyl-ACP reductase and an aldehyde decarbonylase from cyanobacteria have been used to convert fatty acyl-ACP to aldehyde, followed to alkane and alkene for the first time in *E. coli* [104]. In another method, different chain lengths and branched alkanes were synthesized by modification of FFA pools coupled with aldehyde decarbonylase from *Nostoc punctiforme*, FFA were converted into aliphatic n- and iso-alkanes/alkenes of various chain lengths in engineered *E. coli* [6]. Besides long chain alkanes, short chain alkanes were synthesized by combining several steps. Firstly, short chain FFA was accumulated by genetic modification. Next, the endogenous fatty acyl-CoA synthetase, fatty acyl-

CoA reductase from *Clostridium acetobutylicum* and fatty aldehyde decarbonylase from *Arabidopsis thaliana* converted short chain FFA into short chain alkanes. Finally, up to 580.8 mg/L of short chain alkanes were produced in the engineered *E. coli* [7].

Although studies in heterologous expression fatty acyl-ACP/CoA reductase (FAR) and aldehyde decarbonylase enzymes in yeast is far more limited than in bacteria, there has been great progress in using yeast for alkane production lately. First, *S. cerevisiae* was demonstrated to synthesize the very long chain alkane by introducing an alkane biosynthesis pathway from plant *Arabidopsis thaliana.* Co-expression of CER1 and CER3 led to very long chain alkane synthesis, including mainly C_{27}, C_{29} and C_{31} alkanes. The additional expression of the cytochrome b5 isoforms CYTB5s enhanced alkane production by providing cofactors [75]. In another study to explore *S. cerevisiae* for long chain alkane production, it was indicated that the difficulty in producing alkane in *S. cerevisiae* was due to the lack of a compatible cytosol redox partner that was required by the aldehyde deformylating oxygenase (ADO) enzyme in the yeast strain. When the hexadecenal dehydrogenase Hfd1 was deleted and an *E. coli* ferredoxin (EcFdx)/ferredoxin reductase (EcFpr) redox system was expressed in yeast, co-expression of an alkane biosynthesis pathway (consisting of a SeFAR from *S. elongates* and a SeADO from *S. elongatus*) led to alkanes production of 22 μg/gWD, including tridecane, pentadecane and heptadecane [105]. To improve the efficiency from FFA to alkane, another pathway including *M. marinum* carboxylic acid reductase (MmCAR), SeADO from *S. elongatus* and NpADO from *N. punctiforme* were expressed in *Δhfd1Δpox1Δadh5* background. 4'-phosphopantetheinyl transferase NpgA from *Aspergillus nidulans* was also expressed to activate MmCAR. Combining all the strategies, an alkanes production of 0.8 mg/L were achieved [15]. In a proof-of-principle study using *S. cerevisiae* for producing medium- and long-chain alkane, co-expression of α-dioxygenase (αDOX) from *Oryza sativa* (rice) and a cyanobacterial aldehyde deformylating oxygenase from *Synechococcus elongatus* PCC 7942 in a FFA-overproducing strain BY4741 *Δfaa1Δfaa4* gave an alkane production of 73.5 μg/L, including 42.4 μg/L tetradecane and 31.1 μg/L hexadecane [74].

In a recent effort on engineering yeast for terminal alkene production, a total 54.5 μg/L terminal alkenes with different chain lengths from C11 to C19 were produced by expressing a codon-optimized cytochrome P450 fatty acid decarboxylase $OleT_{JE\text{-}CO}$ from *Jeotgalicoccus sp.* ATCC8456. Moreover, the production titer was increased 7-fold by improving the availability of the precursor fatty acids, which was done by deleting two main fatty acyl-CoA synthetases *FAA1* and *FAA4*. In addition, overexpression of HEM3 and triple-deletion of CTT1, CTA1 and CCP1 accumulated more endogenous heme and hydrogen peroxide, which further enhanced the production another 23%. Combining with gene expression tuning and bioreactor process optimization, the alkene production was improved 67.4-fold to the titer of 3.7 mg/L [78].

3.5. Fatty aldehydes production

Besides acting as intermediates for alcohol or alkane biosynthesis, microbial synthesized aldehydes have many industrial uses, such as flavors and fragrances, precursors of pharmaceuticals and pesticides [106]. In addition to the acyl-ACP/CoA reductase, *M. marinum* carboxylic acid reductase (MmCAR) [15] and α-dioxygenase (αDOX) [72] have been used in convert fatty acid into

aldehyde. To circumvent the toxicity of aldehyde to cell, in vitro synthesis could be carried out by purified heterologous expressed *Nocardia iowensis* CAR. However, its activity is inhibited by forming the co-product pyrophosphate. It was demonstrated that the rate and yield of aldehyde synthesis was greatly improved by pairing an inorganic pyrophosphatase (P_{paEc}) with CAR [107]. To better predict the activity of CAR, different CARs were characterized for diversifying aldehyde production [108].

Besides the above mentioned fuels and chemicals, other chemicals traditionally produced from petroleum or plant oil can also be made from microbial fatty acids, such as R-3-hydroxybutyric acid [109,110], polyhydroxyalkanoates [111,112], poly-unsaturated fatty acids (PUFA) [113] and flavonoid [114].

4. Solutions to Enhance the Productivity

S. cerevisiae has showed its advantages in the production of fatty acid-derived products. However, it does not accumulate large quantities of fatty acids, or fatty acid derived products naturally. The prerequisite for efficiently producing fatty acid-derived products in *S. cerevisiae* is to improve the productivity and metabolic performance of the engineered strain. For this, the precursor and co-factor supply, enzyme activities, toxicity and competing pathway, all have been found to be limiting high production titers. Thus, necessary and efficient genetic manipulations need to be carried out to solve the bottlenecks and enhance the productivity.

4.1. Enhancement of precursor and co-factor supply

To enhance the supply of the direct precursor fatty acid, the precursors and co-factors required for fatty acid biosynthesis, acetyl-CoA, malonyl-CoA, ATP and NADPH, firstly need to be enhanced [19]. In *S. cerevisiae*, there are several source of acetyl-CoA: it can be produced by the pyruvate dehydrogenase (PDH) pathway in the mitochondria or by the pyruvate-acetaldehyde-acetate (PDH bypass) pathway in the cytosol. In the mitochondria, pyruvate dehydrogenase complex is involved in catalyzing the overall conversion of pyruvate to acetyl-CoA [115]. In the cytoplasm, ACS enzymes Acs1p and Acs2p are involved in the reaction of catalyzing acetate to acetyl-CoA [116].While in oleaginous microorganisms, there is an additional source of acetyl-CoA: the excess citrate exported from the TCA cycle out of the mitochondrion can be cleaved by ACL in the cytosol [117]. In addition, the cytosolic acetyl-CoA is limited by the Crabtree effect with glucose as carbon source, because the limitation in respiratory capacity lead to the majority of the glycolytic flux is directed toward ethanol and glycerol [118].

It has been shown that engineering the PDH bypass can enhance a wide range of acetyl-CoA-derived products by overproducing acetyl-CoA. Isoprenoids production in yeast was increased by overproduction of acetaldehyde dehydrogenase (ALD6) and a *Salmonella enterica* acetyl-CoA synthetase variant (ACS_{SE}^{L641P}) [119]. The production of a-santalene in *S. cerevisiae* was improved 4-fold by introducing a plasmid pIYC08 containing genes *ADH2* encoding alcohol dehydrogenase, *ALD6* encoding acetaldehyde dehydrogenase, ACS_{SE}^{L641P} encoding acetyl-CoA synthetase and *ERG10* encoding acetyl-CoA acetyltransferase [120]. Furthermore, the production of polymer

polyhydroxybutyrate (PHB) was improved more than 16 times by co-transformation of a plasmid containing the bacterial PHB pathway genes together with the same plasmid pIYC08 [109]. With same method, butanol was improved 6.5-fold by co-expression of the butanol pathway with pIYC08 in the *MLS1* or *CIT2* deletion strains [121].

Overexpression of ACL was also shown to increase the acetyl-CoA support towards de novo lipid synthesis in non-oleaginous [117]. Disrupting the citrate turnover and overexpressing a heterologous ACL increased the acetyl-CoA supply, which led to a 1.92-fold increase in C16:1 and a 1.77-fold increase in C18:1 intracellular long chain fatty acids [122]. In another example, expression of ACL from *A. thaliana* and *Y. lipolytica* in a yeast strain engineered for overproducing fatty alcohols, improved the production 55% and 136% respectively [14].

Acc1 catalyzed malonyl-CoA formation is the rate-limiting step for fatty acid synthesis in *S. cerevisiae* [83]. Overexpression of *ACC1* could increase the malonyl-CoA level thus enhance fatty acid-derived biofuels and chemicals production [14,82]. However, no more than 2-fold improvement was observed in most studies just by overexpressing *ACC1*. Besides increasing the expression level of the genes involved in fatty acid synthesis, the enzyme activity also can be controlled at protein level. For example, Acc1 is repressed by Snf1 protein kinase [123]. It was found that by introducing Ser659 and Ser1157 mutations in phosphorylation sites of Acc1, the posttranslational repression was abolished. Acc1 activity was enhanced and total fatty acid content was increased. Overexpression of $ACC1^{S1157A,\ S659A}$ in a strain harboring wax ester synthase gene (*ws2*) increased FAEEs three-fold to 15.8 mg/L, as compare with FAEE-producing control strain that only holds the *ws2* gene [123]; while overexpression of $ACC1^{wt}$ could only increase FAEE production about 20%. In addition, malonyl-CoA can also be directly synthesized from malonate by malonyl-CoA synthetase (MCS). Overexpression of a plant MCS gene *AAE13* in *S. cerevisiae* has been shown to enhance the lipid and resveratrol production 1.6-fold and 2.4-fold respectively [124].

Besides the precursors supply, the limited co-factor supply also contributed to the low production of fatty acid-derived products. FAS requires two molecules of NADPH in the two reduction steps of the carbon chain elongation cycle. In the cytoplasm of yeast, NADPH is mainly produced by the pentose phosphate pathway through $NADP^+$ dependent dehydrogenase, including glucose 6-phosphate dehydrogenase, decarboxylating P-gluconate dehydrogenase, and NADPH isocitrate dehydrogenase. $NADP^+$ also can be generated by NAD kinase, which may play a role in establishing NAD/NADP ratios. Malic enzyme is also showed to provide NADPH by catalyzing the reaction: Malate + $NADP^+$→pyruvate + NADPH. Malic enzyme has been found in most oleaginous microorganisms, and has been suggested as controlling activity for lipid production in *Mucor circinelloides* and *Aspergillus nidulans* [125,126]. Besides, NADPH can also be provided by aldehyde dehydrogenase family [127,128]. In a study, overexpression of a bacterial $NADP^+$-dependent glyceraldehyde-3-phosphate dehydrogenase (*gapN*) enhanced NADPH supply and further improved FAEEs production [96].

4.2. Improvement of enzyme activities

Lots of enzymes have been exploited and used to construct novel biofuels and chemicals synthesis pathways in microbes. The activities of these key enzymes determine the forward driving

force, which can direct carbon flux to the desired end products. However, most of them are not very efficient, therefore it is difficult to achieve high production levels. Intensive efforts have been put in selecting efficient new enzymes and improving enzyme activity.

For example, expression of different thioesterases resulted in different FFA production levels in yeast [67,82,83]. The activity of CAR on different carboxylic acids were also investigated [129]. Based on the important reaction catalyzed by aldehyde decarbonylase, lots of studies have been carried out to investigate the mechanism and improve its low activity. To accurately describe the reaction it catalyzed, it is redesignated as aldehyde deformylating oxygenase (ADO) [73]. Its activity is found to be inhibited by hydrogen peroxide (H_2O_2). The inhibition can be prevented by fusing a catalase to its N-terminal, which can convert H_2O_2 to O_2 [130]. Moreover, there is study about improving the ligand specificity by site-mutation [131], and improving catalyzing efficiency by fusing to alternative electron transfer systems [132].

4.3. Improvement of the strain tolerance

The toxicity and cellular stress caused by accumulation of fatty acid-derived products limit the achievable production titers in the engineered strains [17,106,133]. For example, high expression of heterologous wax ester synthase genes and deletion of the competing pathways influenced physiological and the transcriptional status of the strain. The cellular stress caused by genetic modification and FAEE production had negative effect on cell growth [134]. Similarly, it has been shown that genetic modifications for FAA production caused cellular stress which had negative effect on cellular growth. The up-regulation of proteins involved in stress response and antioxidant proved the toxicity. Further direct the metabolic flux toward FFA by expression of heterologous efficient thioesterase could built new balance and down regulated the stress response and antioxidants proteins [135].

To study and overcome the toxicity of alkanes to *S. cerevisiae*, the role of efflux pumps Snq2p and Pdr5p on alkane exportation and tolerance were investigated at a transcriptional level in *S. cerevisiae* [136], and they were demonstrated to be involved in decane and undecane export and tolerance. Heterologous expression of ABC2 and ABC3 transporters from *Y. lipolytica* were demonstrated to significantly improve the tolerance of *S. cerevisiae* against decane and undecane [137]. Besides efflux pumps, the pleiotropic drug resistance (Pdr) transcription factors Pdr1p and Pdr3p were also exploited in alkane tolerance study. Expression of the point mutations of *PDR1* (F815S) and *PDR3* (Y276H) in *S. cerevisiae* BY4741 *Δpdr1Δpdr3* (BYL13) led to the highest tolerance to C10 alkane, and the expression of wild-type *PDR3* in BYL13 resulted in the highest tolerance to C11 alkane. Alkane transport assays indicated that the high tolerance was due to reduction of intracellular alkane accumulation, which was resulted from higher export and lower import of alkane [138].

4.4. Elimination of competing pathways

Elimination of competing pathways is one of the main approaches to enhance the production of aim products. For fatty acid derived products accumulation in yeast, a lot of such methods have been

adopted. For example, deletion of acyl-CoA oxidase *POX1* in β-oxidation and deletion of aldehyde dehydrogenase Hfd1 have been shown to increase the fatty acid synthesis [72]. For the production of fatty alcohols, in order to identify the main competing pathways for aldehyde intermediates, 17 putative aldehyde reductases (ALRs) and/or alcohol dehydrogenases (ADHs) was single deleted. It was demonstrated that deletion of *ADH5* increased alkane production and decreased alcohol accumulation [15].

Furthermore, so many highly active endogenous aldehyde reductases (ALRs) existed *E. coli* can convert aldehydes to alcohols and limit the alkane production. In order to improve aldehyde and alkane production, in one study, 13 known ALRs were collectively deleted to reduce endogenous ALR activity [139].

Besides all the solutions mentioned above, control the host regulatory network and culture conditions optimization are also very important in the control of fatty acid-derived products biosynthesis. For example, by engineered FapR, a transcription repressor that specifically senses malonyl-CoA level, a regulatory network was built to dynamically regulate the critical enzymes involved in malonyl-CoA metabolism and efficiently redirect carbon flux toward fatty acid accumulation [140].

5. Conclusions

In conclusion, studies have demonstrated the ability of *S. cerevisiae* in fatty acid-derived biofuels and chemicals production. Based on the progress in utilizing yeast fatty acid biosynthetic machinery to produce various valuable products, we could see its great potential as an excellent industrial biosynthesis platform. To be competitive with current petroleum and plant oil derived chemicals, fatty acid metabolic pathway and heterologous synthesis pathways need be further optimized to reach high yields. To this aim, different strategies need to be combined, including precursor and co-factor supply enhancement, enzyme activities optimization, strain tolerance improvement, competing pathways elimination and culture conditions optimization. Together with new enzymes discovery and strategies development, more efficient cell factory platforms will be constructed in the future.

Conflict of Interest

The authors declare that they have no conflict of interest.

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