Oleaginous Fungi in Biorefineries

Shousong Zhu, Hainan University, Haikou, China
Gregory Bonito, Michigan State University, East Lansing, MI, United States
Yinhua Chen, Hainan University, Haikou, China
Zhi-Yan Du, Michigan State University, East Lansing, MI, United States

Introduction

Many unicellular microorganisms including yeasts, fungi, microalgae, and to a lesser extent bacteria (**Fig. 1**), can produce intracellular edible oils under normal and specialized conditions (Papanikolaou and Aggelis, 2011). Most of these organisms can accumulate microbial lipids, or single-cell oils (SCOs), to 20%–90% (w/w) of their dry cell biomass. For example, fungi in the genus *Mortierella* and *Umbelopsis* can accumulate lipids at concentrations that exceed 86% of their dry weight (Meng *et al.*, 2009). Microbial lipids can be produced using low-priced organic materials, including waste-streams from the food industry, as growth substrate. The fatty acid of microbial lipids is very similar to the conventional vegetable oils in type and composition (Madani *et al.*, 2017). Furthermore, microbial lipids have many potential applications, including human food additives, nutraceuticals, pharmaceuticals, cosmetics, biopolymers and feed ingredients for aquaculture, and as an alternative feedstock for the production of biofuel (Lewis *et al.*, 2000). Due to the reduced availability of cultivable land and increasing human population growth, producing lipids via traditional methods will not satisfy the rapidly growing global demand. Therefore, microbial lipids accumulated from microorganisms, especially oleaginous fungi, are considered as a vital and renewable oil resource and have been regarded as an alternative to animal and plant lipids in recent years, given their unique characteristics and functions in energy, chemical, and food industries (Huang *et al.*, 2017). The production of microbial lipids is particularly attractive when low or negative cost substrates are used as the feedstock.

Oleaginous Fungi and Their Advantages for SCO Production

Fungi consist of a large group of diverse species and represent their own Kingdom of life. Fungi have conquered almost all habitats on the planet, from glacial deep seawater to hot and dry desert, and associate with single prokaryotes, eukaryotic mammals and plants (Gupta *et al.*, 2013). Some fungi are biotrophic and cause negative impacts on native organisms, such as the rice blast pathogen *Pyricularia oryzae* (previously known as *Magnaporthe oryzae*) (Zhang *et al.*, 2015). However, many fungi are beneficial to their hosts, such as mycorrhizas that provide their plant hosts with mineral nutrition. Some filamentous fungi produce high amounts of lipids, and are called oleaginous fungi. Examples of oleaginous filamentous fungi (**Fig. 1**) include *Mortierella alpina* (Wang *et al.*, 2011), *Umbelopsis isabellina* (previously known as *Mortierella isabellina*) (Harde *et al.*, 2016), *Mucor circinelloides* (Song *et al.*, 2001), and *Cunninghamella echinulate* (Fakas *et al.*, 2009b). These species have attracted much attention for their high yield of long-chain polyunsaturated fatty acids (PUFAs). With the advancement of fermentation technology, microbial lipids (primarily from fungi and yeast) can be produced in quantities equivalent to acres of agricultural land (Alvarez and Steinbüchel, 2002), and SCO produced by oleaginous fungi could contribute to meeting human demands for industrial oils.

Biorefineries are facilities that produce multiple products including chemicals, biofuels, and are specialized at making valuable bioproducts from waste residues. Applications of oleaginous fungi in biorefineries have become more attractive given their renewable, resource-efficient, and environment-friendly features (Peng and Chen, 2008), as well as their versatility and high efficiency in utilizing complex and diverse substrates (Ferreira *et al.*, 2012). Overall, the main advantages of oleaginous fungi are shown as follows: (1) High yield of lipid content (up to 90%) and high-value compounds such as PUFAs. For example, *U. isabellina* was able to produce large amounts of cellular lipids amounting to over 80% of their dry cell biomass (Chatzifragkou *et al.*, 2010; Gao *et al.*, 2013). (2) Outstanding lipid profiles for high-quality biodiesel. Not all biological lipids are suitable for biodiesel production. In fact, only saponifiable lipids and free fatty acids can be turned into fatty acid methyl esters (FAMEs), the main content of biodiesel. Most fungus-derived microbial lipids are suitable for high-quality biodiesel production. For example, 98% of the lipids produced by *M. circinelloides* were saponifiable lipids and free fatty acids, which could directly be acid-catalyzed into biodiesel (Vicente *et al.*, 2010). Microbial lipids produced by the filamentous fungus *Aspergillus oryzae* had appropriate fatty acid profiles for biodiesel production: 11.6% of palmitic acid, 15.6% of palmitoleic acid, 19.3% of stearic acid, 30.3% of oleic acid, 5.5% of linolenic acid, and 6.5% of linoleic acid (Muniraj *et al.*, 2013). (3) Easy to form pellets under submerged fermentation that can reduce the viscosity of the fermentation culture and it is easy and cheap to harvest (Koike *et al.*, 2001; Reis *et al.*, 2014; Xia *et al.*, 2014; Xia *et al.*, 2011); (4) Flexibility of various carbon sources for lipid production including *e.g.*, glycerol, wheat straw, tomato waste hydrolysate, potato processing wastewater, corn stover, brewery waste, and glucose (André *et al.*, 2010; Fakas *et al.*, 2007; Fang *et al.*, 2016; Gema *et al.*, 2002; Muniraj *et al.*, 2013).

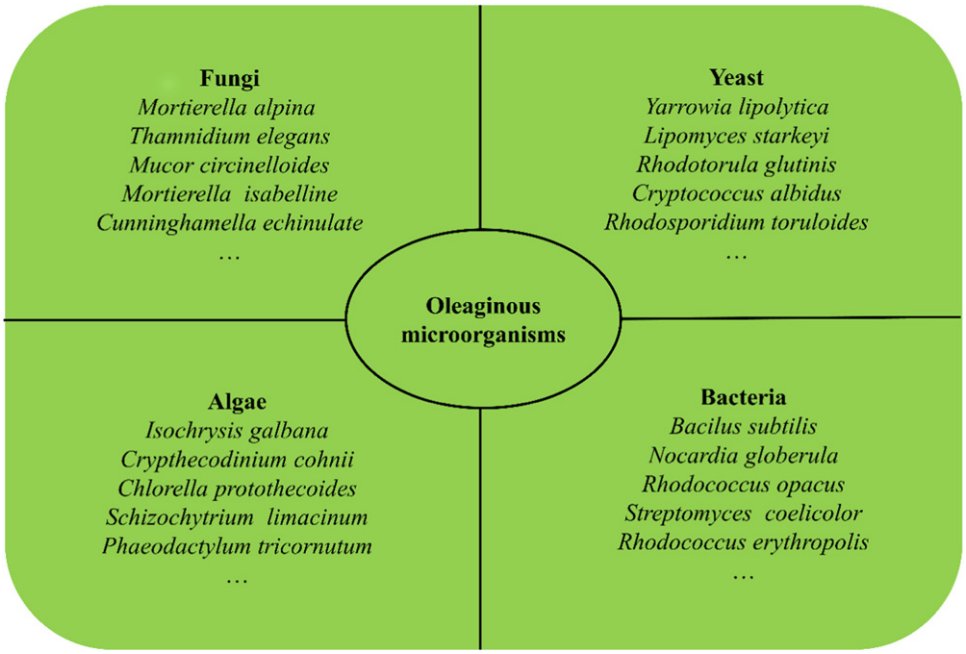

Fig. 1 Common oleaginous microorganisms.

Limiting Factors of Large-Scale SCO Production

Although microbial lipids from oleaginous fungi have promising potential for many applications, their high production cost compared to plant and animal-derived lipids still limit the broader use of microbial lipids (Zhao *et al.*, 2011). There are three major costs associated with producing microbial lipids: carbon and nutrient source, fermentation, and post-processing operations associated with separating lipids from oleaginous organisms. According to the previous study, the cost of feedstocks or carbon sources can account for up to 75% of the total costs for producing microbial lipids (Subramaniam *et al.*, 2010). Thus, the production cost could be dramatically reduced when cheaper feedstocks or waste residues are used for microbial lipid production. During recent years, a series of new microbial lipid processing techniques have been developed and used to cultivate oleaginous fungi using agricultural residues as the carbon source to lower costs and increase sustainability (Carota *et al.*, 2018; Economou *et al.*, 2011; Fang *et al.*, 2016; Zhang and Hu, 2012; Zheng *et al.*, 2012). For example, the fungus *Aspergillus niger* accumulated 41%–57% of lipid when it was cultured on biodiesel-derived waste glycerol (André *et al.*, 2010).

Many studies have been carried out to reduce downstream processing costs, one of the major obstacles that need to be solved to improve the economic viability of producing microbial-derived lipids, especially for large-scale commercialization (Ochsenreither *et al.*, 2016). It has been reported that the filamentous fungus *U. isabellina* formed pellets with an average diameter of 0.11 mm when cultured on non-detoxified liquid hydrolysate (Zheng *et al.*, 2012), which makes the downstream harvesting process easier. Some feasible ways have been reported to induce fungal pelletization by adding $CaCO_3$ or adjusting the pH (Liao *et al.*, 2007; Xia *et al.*, 2011), which offered a feasible direction for low-cost harvesting, a major step during downstream processing.

Lipid Synthesis Mechanism of Oleaginous Fungi

Biochemistry of Lipid Accumulation

Insights on the lipid biochemistry and physiology in oleaginous microorganisms can promote both basic research and industrial applications. A better understanding of the lipid synthesis mechanism in oleaginous microorganisms will help us improve lipid yield and acquire the desired fatty acid profiles. Previous studies have elucidated the biochemical mechanisms of lipid accumulation in various microorganisms, including key enzymes, regulation of lipid accumulation, and key intermediates in lipid biosynthesis (Alvarez and Steinbüchel, 2002; Burton *et al.*, 2005; Hao *et al.*, 2014; Ratledge, 2014; Shuib *et al.*, 2018; Tang *et al.*, 2016; Zhao *et al.*, 2016). There are similarities in the lipid accumulation mechanisms among different oleaginous organisms. Regarding the oleaginous microorganisms, lipid accumulation often happens during the starvation of nutrients including nitrogen (N), phosphorus (P), sulfur (S), vitamins, and metals such as zinc (Zn), iron (Fe), and magnesium (Mg) (Laoteng *et al.*, 2011; Madani *et al.*, 2017). Significant lipid accumulation rarely occurs under enriched nutrient conditions. However, the cells react to the limits of a key nutrient, *i.e.*, N, P, K, Mg, S and Fe that are required for cell proliferation, and they will enter into lipid storage

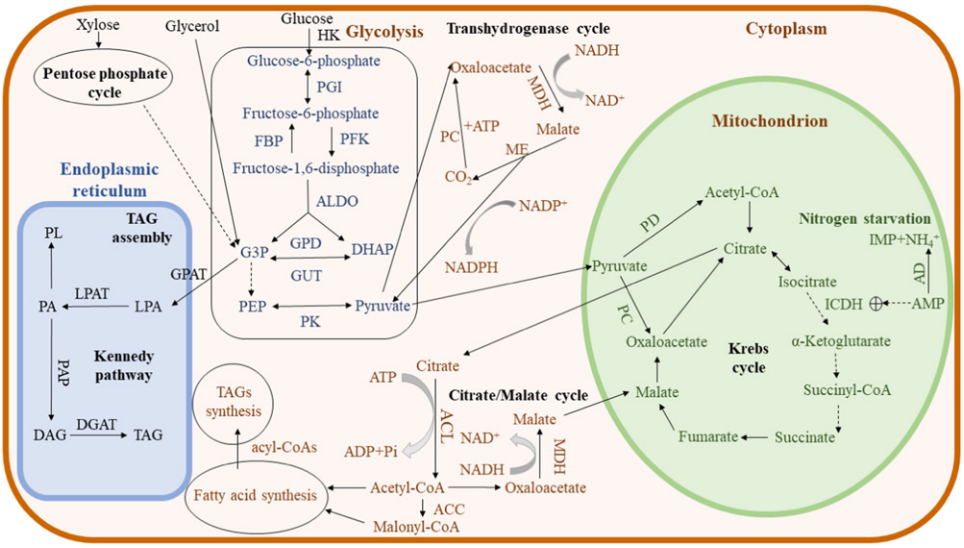

Fig. 2 Outline of the main biochemical process causing lipid accumulation in oleaginous fungi. AD, adenosine deaminase; ALDO, aldolase; DHAP, dihydroxyacetone phosphate; FAS, fatty acid synthase; FBP, fructose-1,6-biphosphatase; G3P, glycerol-3-phosphate; GPD and GUT, isoforms of G3P dehydrogenase; HK, hexokinase; ICDH, isocitrate dehydrogenase; MDH, malate dehydrogenase; ME, malic enzyme; PC, pyruvate carboxylase; PD, pyruvate dehydrogenase; PEP, phosphoenolpyruvate; PFK, phosphofructokinase; PGI, glucose-6-phosphate isomerase; PK, pyruvate kinase.

phase, in which excess carbon is converted to lipid reserves by unique lipid biosynthetic pathways. The limited supplement of nutrients inhibits cell proliferation and the synthesized lipid can be stored in the oil bodies of undivided cells (Alvarez and Steinbüchel, 2002; Beopoulos *et al.*, 2009a,b; Dourou *et al.*, 2018; Ratledge and Wynn, 2002; Shields-Menard *et al.*, 2018).

Among those key nutrients, N-limitation is the most important factor for lipid accumulation. The fatty acids for microbial lipids from oleaginous fungi are typically synthesized from acetyl-CoA with a reversed β-oxidation in the cytoplasm. As shown in **Fig. 2**, when the N supply is limited, the activity of adenosine monophosphate (AMP) deaminase will be upregulated, which breaks down AMP, an essential cofactor of isocitrate dehydrogenase (ICDH), and releases ammonia to N-starved cells. ICDH is a key enzyme in the citric acid cycle. It will become inactive in the absence of N and unable to convert isocitrate to α-ketoglutarate. As a consequence, the tricarboxylic acid cycle (TCA) is blocked at isocitrate due to the declined concentration of cofactor AMP along with the increase in AMP deaminase activities. Subsequently, the accumulated citrate is transferred from the TCA cycle and diverted into the cytoplasm from mitochondria via citrate-malate translocase. In the cytosol, the citrate is catalyzed and decomposed into acetyl-CoA and oxaloacetate by ATP citrate lyase (ACL). The latter is then converted into malate by malate dehydrogenase. The malate could be translocated to the mitochondria by citrate-malate translocase or converted into pyruvate via the malic enzyme (ME) with the production of NADPH and CO_2 during the cytosolic transhydrogenase cycle. Resulting from N limitation, accumulated acetyl-CoA and NADPH enter into fatty acid and triacylglycerol (TAG) synthesis (Jin *et al.*, 2015; Ratledge and Wynn, 2002; Ratledge and Wynn, 2020). In contrast, when the N availability increases, the cell will convert storage lipids into cellular materials (Daum *et al.*, 2007; Ratledge, 2002).

In addition, the ratio of carbon to nitrogen (C/N) in the culture is a crucial factor for lipid accumulation in oleaginous microorganisms. It has been shown that microbial lipid accumulation could be improved by optimizing the process parameters and culture conditions including the C/N ratio, N source content, and oxygen concentration through new process configurations such as solid-state or semi-solid-state fermentation methods (Kosa and Ragauskas, 2011). Increasing the ratio of C/N in culture media significantly improves the lipid yields from oleaginous fungi (Fakas *et al.*, 2007). According to current studies, the optimal C/N ratio depends on the organism, but exceeds 65 and may exceed 100 (Ageitos *et al.*, 2011; Jin *et al.*, 2015). Increasing the ratio of C/N was observed to promote lipid accumulation with longer growing periods in oleaginous fungi (Papanikolaou *et al.*, 2004; Ruan *et al.*, 2012), and there is usually an optimum carbon concentration for each oleaginous fungal species at which lipid accumulation is maximized (Murphy, 1990). Under C/N ratios of 35, 44, and 57, *U. isabellina* ATHUM 2935 accumulated about 36%, 51.2% and 64.3% of lipids, respectively, using rice hull hydrolysate (Economou *et al.*, 2011). *Cunninghamella echinulata* ATHUM 4411 was able to produce ~25% of lipids at a C/N ratio of 78 in an N-limited medium supplemented with xylose. Its lipid content reached a maximum of 57.7% at a higher C/N ratio of 285 (Fakas *et al.*, 2009b). When cultivated in xylose media, *U. isabellina* ATHUM 2935 behaved similarly to *C. echinulata* ATHUM 4411 with a higher lipid yield of 65.5% compared to 57.7% in *C. echinulata* ATHUM 4411 (Fakas *et al.*, 2009b).

Two types of lipid synthesis pathways have been described in oleaginous microorganisms, designated "*de novo*" and "*ex novo*" lipid accumulation (Papanikolaou and Aggelis, 2011). The *de novo* lipid accumulation process is often fermented on hydrophilic materials (sugars and related substrates) with excessive exhaustion in the medium of an essential nutrient (typically N). In contrast, the *ex novo* lipid accumulation pathway is carried out on the hydrophobic materials (oils, alkane, *etc.*) along with the

growth process and does not require N-limiting culture conditions (Huang *et al.*, 2013; Papanikolaou *et al.*, 2001, 2002). The main difference between the two pathways is that the *ex novo* lipid accumulation occurs simultaneously with cell growth and is independent with the nutrient-limiting culture conditions. Each pathway has unique advantages: *de novo* lipid accumulation provides great lipid yield, while *ex novo* lipid accumulation allows modification on the ratio between intracellular and extracellular lipid composition to meet the requirement for different applications (Huang *et al.*, 2013, 2017; Papanikolaou and Aggelis, 2011). The two pathways could be engineered simultaneously. For example, Huang *et al.* (2017) combined "*de novo*" and "*ex novo*" lipid fermentation using corncob acid hydrolysate with soybean oil as a mixed-medium to cultivating oleaginous yeast *Trichosporon dermatis*, which could simultaneously and efficiently utilize both hydrophilic and hydrophobic substrates in the medium.

Lipid Profiles in Oleaginous Fungi

The growth of fungi is usually accompanied by relative changes in the number of lipid fractions and unique types of lipids, as well as in fatty acid composition. Oleaginous fungi accumulate lipids predominantly in the form of TAG. Other accumulated lipids include monoacylglycerols, diacylglycerols, free fatty acids, sterols, sterol esters, and polar lipids (glyco-, sphingo-, and phospholipids) (Khot *et al.*, 2018). TAGs are neutral, insoluble fatty acid tri-esters of glycerol, which possess a higher caloric value than carbohydrates and proteins, and thus provide an abundant reserve with much higher energy via β-oxidization (Alvarez and Steinbüchel, 2002). TAGs represent the predominant storage form of carbon and energy in oleaginous microorganisms. Following phospholipid synthesis during the exponential growth phase, TAGs usually accumulate in the stationary phase when cellular growth is impaired under excess carbon and limited N conditions (Ratledge and Wynn, 2002). Fatty acids in microbial lipids range from lauric acid (C12:0) to docosahexaenoic acid (DHA, C22:6). Palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids are the most common fatty acids in fungi. Among those fatty acids, $\sim$50% are unsaturated fatty acids (Subramaniam *et al.*, 2010).

It has been reported that the fatty acid profiles in microbial lipids are mainly dependent on the species (Xu *et al.*, 2012). For example, in *U. isabellina*, the lipids consist of 49%–54% oleic acid, 24%–35% palmitic acid, 2%–11% linoleic acid, 3.5%–8% stearic acid, 1%–2% palmitoleic acid, and 0.4%–2% γ-linolenic acid (Economou *et al.*, 2010), which is similar to *Microsphaeropsis* sp.: 46.1% oleic acid, 27.3% palmitic acid, 20.0% linoleic acid, 3.5% palmitoleic acid, and 3.0% stearic acid (Peng and Chen, 2008). In contrast, *Aspergillus tubingensis* TSIP9 accumulated 42.7% saturated and 55.3% unsaturated fatty acids including 35.8% oleic acid, 21.5% palmitic acid, 18.8% linoleic acid, and 9.6% stearic acid (Cheirsilp and Kitcha, 2015). *U. isabellina* and *C. echinulate* have the same types of fatty acids, and the composition was independent of the carbon sources. At the beginning of the growth period, linoleic acid (C18:2) and γ-linolenic acid (C18:3) were the most abundant fatty acids. However, oleic (C18:1) and palmitic (C16:0) acids became the key fatty acids after the lipid accumulation over 20% (Fakas *et al.*, 2009a,b).

The content of microbial lipids in fungi also depends on hereditary factors and culture conditions like agitation, pH, temperature, light, and nutrient content (Subramaniam *et al.*, 2010). Meng *et al.* (2009) reported that the oil content and composition in oleaginous fungi were approximate 7%–23% of C16:0, 1%–6% of C16:1, 2%–6% of C18:0, 19%–81% of C18:1, 8%–40% of C18:2, and 4%–42% of C18:3 according to the fungal strains and growth conditions such as pH, temperature, and culture phase. The optimal culture temperature for oleaginous fungi ranges from 20 to 28°C and pH 4–7 is optimal for their growth (Jin *et al.*, 2015). To some extent, metal ions affect the oil content of oleaginous microorganisms. Several studies have been attempted to improve the PUFAs of oleaginous fungi via optimizing different kinds of metal ions in the culture medium. For example, Cu and Zn have a positive effect on the γ-linolenic acid (GLA, C18:3, ω-6) production of *Umbelopsis ramanniana* (Hansson and Dostálek, 1988). In *Cunninghamella* sp., Mg, Fe, and Zn have significant effects on lipid accumulation because the supplement of each ion resulted in a 64%, 43%, and 33% increase in lipid content compared to the cultures deprived with each of these ions, respectively (Muhid *et al.*, 2008). Among the three ions, Zn has the most significant effect on fatty acid content and contributed to a 74% increase in GLA (Muhid *et al.*, 2008). In addition, lipid accumulation and fatty acid compositions could be affected by the carbon sources (*e.g.*, glucose and xylose) in various zygomycete fungi (Dyal *et al.*, 2005; Hansson and Dostálek, 1988; Sajbidor *et al.*, 1988; Somashekar *et al.*, 2003).

Different Substrates for Microbial Lipids Production

Conventional Carbon Substrates

As shown in **Table 1**, various kinds of carbohydrates, such as sucrose, molasses from sugar beet and sugarcane, glucose and dextrins from hydrolyzed starch, whey (a by-product of cheese production), fructose produced from inulin, and some natural hydrolysates like rice straw hydrolysate, tomato waste hydrolysate, wheat straw hydrolysate, sugarcane bagasse hydrolysates, and potato processing wastewater, are raw materials derived from agro-industrial processes, which could be used as substrates for microbial lipids production (Chatzifragkou *et al.*, 2010; Fakas *et al.*, 2008a,b; Muniraj *et al.*, 2013). Several monosaccharides, disaccharides, and carboxymethyl-cellulose (CMC) were tested as carbon sources for microbial lipid production by the filamentous fungus *U. isabellina* (Zeng *et al.*, 2013). Cultivated with C5 (xylose) and C6 (fructose and glucose) monosaccharides, *U. isabellina* was able to produce over 60% cellular lipids of total biomass, compared to the 17% and 41% of lipid using disaccharide sucrose and cellobiose, respectively. Due to the absence of cellulase enzymes, CMC did not stand out as a good substrate for *U. isabellina* with only 5% of lipid produced (Zeng *et al.*, 2013). Glycerol is a common by-product of biodiesel production and it could be used as

Table 1 Lipid production of oleaginous fungi using various carbon sources.

Oleaginous fungi	*Lipid content (%, w w^{-1} CDW)*	*Biomass (g L^{-1})*	*Lipid yield (g L^{-1})*	*Substrate*	*Cultivation time (h)*	*References*
Alternaria sp. DM09	55.10	13.80	7.60	Glucose	240	(Dey *et al.*, 2011)
Aspergillus oryzae	40.00	8.75[a]	3.50	Potato processing wastewater	120	(Muniraj *et al.*, 2013)
A. oryzae A-4	18.15	4.31	39.08[b]	Cellulose	144	(Lin *et al.*, 2010)
Aspergillus tubingensis TSIP9	NA	NA	31.10[b]	Palm pressed fiber	120	(Kitcha and Cheirsilp, 2014)
Colletotrichum sp. DM06	46.08	16.20	7.30	Glucose	240	(Dey *et al.*, 2011)
Cunninghamella echinulata ATHUM 4411	25.00	31.2[a]	7.80	Tomato waste hydrolysate + glucose	300	(Fakas *et al.*, 2007)
	57.70	7.80	4.50	Xylose	192	(Fakas *et al.*, 2009b)
	46.00	15.00	6.90	Glucose	360	(Fakas *et al.*, 2009b)
	25.60	7.80	2.00	Glycerol	340	(Fakas *et al.*, 2009b)
	49.00	8.90	4.40	Glucose	480	(Gema *et al.*, 2002)
	36.30	4.30	1.56	Raw glycerol	135	(Chatzifragkou *et al.*, 2011)
	30.00	12.90	3.90	Commercial glucose	309	(Chatzifragkou *et al.*, 2010)
	32.00	12.10	3.80	Molasses	356	(Chatzifragkou *et al.*, 2010)
Mortierella ramanniana MUCL 9235	37.10	7.30	2.71	Raw glycerol	216	(Chatzifragkou *et al.*, 2011)
Mucor sp. LGAM 365	18.10	5.30	0.96	Raw glycerol	237	(Chatzifragkou *et al.*, 2011)
Thamnidium elegans CCF1465	42.60	6.80	2.90	Raw glycerol	271	(Chatzifragkou *et al.*, 2011)
Trichosporon dermatis CH007	41.50	23.60	15.10	Corncob acid hydrolysate with soybean oil	168	(Huang *et al.*, 2017)
Umbelopsis isabellina	57.34	44.94	78.66[a,b]	Corn stover	222	(Fang *et al.*, 2016)
U. isabellina ATCC 42613	30.00	16.80	5.10	Acid- and alkali-pretreated corn stover hydrolysate	93	(Ruan *et al.*, 2014)
U. isabellina ATHUM 2935	65.50	8.70	5.70	Xylose	216	(Fakas *et al.*, 2009b)
	44.60	27.00	12.04[a]	Glucose	360	(Fakas *et al.*, 2009b)
	53.20	6.20	3.30	Glycerol	264	(Fakas *et al.*, 2009b)
	74.00	13.20	9.90	Commercial glucose	237	(Chatzifragkou *et al.*, 2010)
	61.00	12.10	7.40	Commercial fructose	405	(Chatzifragkou *et al.*, 2010)
	54.00	9.50	5.10	Molasses	150	(Chatzifragkou *et al.*, 2010)
U. isabellina IFO7884	NA	NA	47.9[b]	Soybean hull	8[c]	(Zhang and Hu, 2012)
U. isabellina MUCL 15102	33.20	5.60	1.86	Raw glycerol	168	(Chatzifragkou *et al.*, 2011)
U. isabellina NRRL 1757	66.68	5.98	3.99	Xylose	144	(Zeng *et al.*, 2013)
	48.21	5.84	2.82	Arabinose	144	(Zeng *et al.*, 2013)
	30.23	3.37	1.02	Ribose	144	(Zeng *et al.*, 2013)
	51.16	9.37	4.80	Mannose	144	(Zeng *et al.*, 2013)
	49.14	8.17	4.01	galactose	144	(Zeng *et al.*, 2013)
	62.50	6.09	3.82	Fructose	144	(Zeng *et al.*, 2013)
	66.50	8.69	5.77	Glucose	144	(Zeng *et al.*, 2013)
	40.87	5.79	2.36	Cellobiose	144	(Zeng *et al.*, 2013)
Zygorhynchus moelleri MUCL 1430	42.40	3.70	1.57	Raw glycerol	192	(Chatzifragkou *et al.*, 2011)

[a]Calculated from the available data.
[b]Measured in 1 mg lipid produced per 1 g dry substrate.
[c]Measured in week.
Abbreviations: CDW, cell dry weight; NA, not available.

the carbon source for oleaginous fungi. Chatzifragkou *et al.* cultivated 15 strains of fungi and yeast with glycerol and the tested stains accumulated 18%–43% (w/w) cellular lipids (Chatzifragkou *et al.*, 2011).

Lignocellulosic Biomass

Lignocellulosic biomass including wood, grass, forestry waste, and agricultural residues, is considered as the most abundant and renewable organic materials (Palmqvist and Hahn-Hägerdal, 2000). Plant-derived lignocelluloses consist of cellulose (35%–50%, dry weight), hemicelluloses (20%–35%) and lignin (10%–25%) (Huber and Corma, 2007; Sun and Cheng, 2003), which have been tested as a suitable feedstock for microbial lipids and biodiesel production because of their abundance and low cost.

Lignocellulosic polymers need to be pretreated and hydrolyzed into readily fermentable sugars by a series of lignocellulolytic enzymes, such as cellulases and hemicellulases (Dyk and Pletschke, 2012). After that, sugars could be utilized by various oleaginous microorganisms (Kitcha and Cheirsilp, 2014). The current challenge for using lignocellulosic biomass includes the high expense of enzymes, and the rate of hydrolysis that may be inhibited by the substrate (sugar), and productivity of microorganisms.

To date, many oleaginous fungi have been demonstrated to utilize lignocellulosic biomass. *U. isabellina* yields 16.8 g L^{-1} of biomass with 5.1 g L^{-1} of lipids (30%, w/w), when cultured on acid- and alkali-pretreated corn stover hydrolysate (Ruan *et al.*, 2014). Two endophytic fungal isolates, *Colletotrichum* sp. (DM06) and *Alternaria* sp. (DM09), produced 68.2 mg gds^{-1} (per gram dry substrate) and 60.32 mg gds^{-1} lipid using rice straw and wheat bran under solid cultivation (Dey *et al.*, 2011). Filamentous fungi are considered as strong cellulolytic enzymes secreting strains in solid-state fermentation conditions (Singhania *et al.*, 2009). For example, *A. tubingensis* TSIP9 could convert palm pressed fiber and palm empty fruit bunches into lipid with the highest production of 31.1 $\pm$ 1.7 or 37.5 $\pm$ 2.2 mg gds^{-1}, respectively. This species produces diverse cellulolytic enzymes with high activity, yet the productivity of cellulase and xylanase can be improved by alkaline pretreatments (Kitcha and Cheirsilp, 2014). In *A. oryzae*, starchy waste substrates were used as the carbon source for microbial lipid production without the need for external amylase. This is because the fungus could secrete amylase and accumulate lipids simultaneously (Muniraj *et al.*, 2013). Direct bioconversions by fungal isolates efficiently reduces time, energy, and costs associated with the enzyme digestion and hydrolysis steps, which are promising for microbial lipid production using lignocellulosic biomass.

The Main Applications of Microbial Lipids From Oleaginous Fungi

The potential applications of oleaginous fungi in biorefineries have been intensively studied in recent years (**Fig. 3**).

Biodiesel

Over the past few decades, fossil fuels, such as petroleum, natural gas, and coal, have been major and essential energy sources consumed globally. However, gas emissions from fossil fuels are harmful to the environment, particularly due to their greenhouse gas contribution to the atmosphere that is the main driver of climate change. Moreover, fossil fuels are inherently finite and they are non-renewable resources in a diminishing process (Wang and Wan, 2009). In fact, many studies have suggested that petroleum reserves will be extracted within 50 years, natural gas within 65 years, and coal within 200 years at the present rate of consumption (Soetaert and Vandamme, 2009). The instability of crude oil supplies and the volatility of prices have further sparked widespread interest in alternative energy sources. With the rapidly increasing global population and the decline of fossil fuel availability,

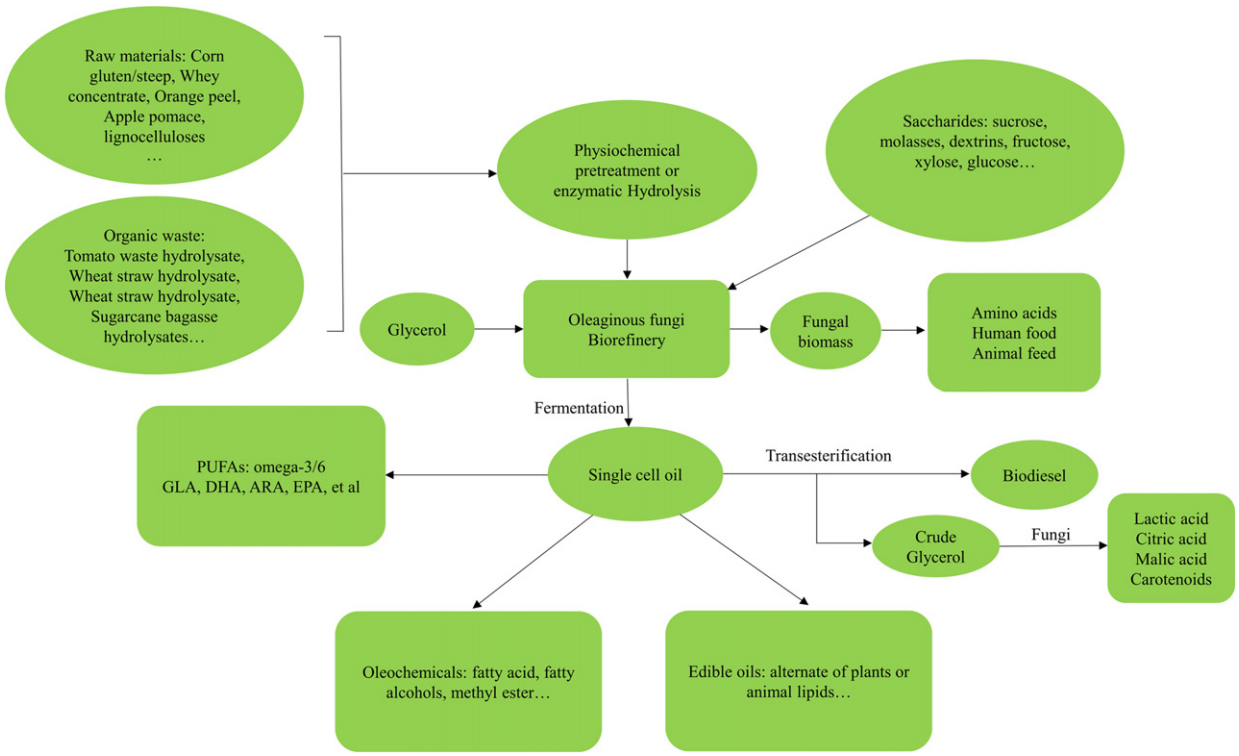

Fig. 3 Schematic diagrams of oleaginous fungi in biorefineries.

traditional methods of producing fuels will not satisfy increasing demands. Thus, it is imperative to develop and utilize sustainable and renewable energy sources. At present, biofuels such as bioethanol and biodiesel are the primary alternative fuels. Compared to bioethanol, biodiesel has several significant advantages: (1) Higher energy density than carbohydrates-based fuel. Biodiesel has an energy density of at least 25% higher than that of ethanol (Durrett *et al.*, 2008). (2) Higher net positive energy balance. For example, soy oil yields 93% more energy than the total energy invested for the production, while the number of corn starch, a major feedstock of bioethanol is 25% (Hill *et al.*, 2006). (3) Lower greenhouse gas emissions. Compared to fossil fuels replaced by biofuels, biodiesel reduced 41% of greenhouse gas emissions and bioethanol reduced them by 12% (Hill *et al.*, 2006). (4) Biodiesel can be used directly for efficient diesel engines and the storage and transport are less expensive compared to ethanol (CharlesDismukes *et al.*, 2008; Durrett *et al.*, 2008). Thus, biodiesel has become a focus of renewable energy research (Demirbas, 2007; Du and Benning, 2016).

Biodiesel is made from monoalkyl esters of long-chain FAMEs produced by transesterification of plant, microbial and algal oils or animal fats. Technically, biodiesel has many advantages including its renewable and non-toxic features, ease of production, good flashpoint, and biodegradability (Meng *et al.*, 2009). More than 95% of the conventional feedstocks for biodiesel production are edible oils including plant oils or animal fats such as palm oils, soybean oils, rapeseed oils, and castor oils, uses which compete with the food industry (Alptekin *et al.*, 2014; Felizardo *et al.*, 2006; Navas *et al.*, 2018; Predojević, 2008; Zabaruddin *et al.*, 2019). Moreover, these conventional feedstocks and raw materials are expensive to produce, which has become the main economical challenge for biodiesel (Srinivasan, 2009). Therefore, it is necessary to find alternative cheap non-edible oil sources and waste feedstock for biodiesel production. In order to lower costs and reduce the environmental impacts of oil-based raw materials, increasing effort has been made on the research of microbial oils. The long-chain fatty acids from microorganisms including oleaginous microalgae, bacteria, and fungi are enriched with energy that makes them ideal precursors for biodiesel (Zhang *et al.*, 2011; Li *et al.*, 2008). Although photosynthetic microalgae have exclusive advantages by utilizing sunlight and carbon dioxide for lipid production, algal biodiesel is facing problems such as high cost of photobioreactors and limitation of large-scale outdoor cultivation (Khot *et al.*, 2018). In contrast, single-cell oils of oleaginous fungi are not affected by light, seasons nor climate, and are promising sustainable feedstocks for biodiesel production (Meeuwse *et al.*, 2013). Further, the content of fatty acid influences the saponification number and iodine value of some specific lipids, and has an impact on the quality of biodiesel, such as cetane number, oxidative stability, the heat of combustion, cloud point, and lubricity (Knothe, 2009). As mentioned above, fatty acid profiles of oleaginous fungi are dominated by myristic, palmitic, stearic, oleic, linoleic, and linolenic acid, all of which could be turned into biodiesel by transesterification reaction (Ruan *et al.*, 2012). The heterotrophic cultivation of oleaginous fungi has many excellent characteristics for industrial biofuel production compared to other oleaginous microorganisms, although there are still many difficulties associated with the industrial production of fungal oils.

The goal of fungal fuels is to develop high quality, low cost, and environmentally-friendly products, which can replace at least a portion of the conventional fuels. Oleaginous fungi, especially yeasts, are efficient in lipid synthesis and have been considered by the biodiesel industry to replace the traditional fossil fuel in the future. Nonetheless, production costs of microbial lipids are still a major problem that restricts their large-scale industrial application. Cheap carbon sources should be developed for the cultivation of those oleaginous fungi. In fact, many alternative economical and sustainable substrates like nongrain plants, agricultural and forest residues, lignocellulosic biomass, food waste, and other waste materials, were used for biodiesel production. For example, using corncob waste liquor as the carbon substrate, a filamentous species of *Aspergillus* was able to produce a high amount of lipids (22.1%; 2 g dry biomass; 48 h), which was ideal for biodiesel production due to its high fraction of saturated fatty acids and other appropriate fuel properties (acid number: 0.40 mg KOH per g of acid; iodine value: 11 g I_2 per 100 g oil; density: 0.8342 g cm^{-3}) (Subhash and Mohan, 2011). Bagy *et al.* (2014) combined fermentative oleaginous fungi with hydrogen-producing bacterium *Clostridium acetobutylicum* ATCC 824 as an integrated system to maximize the biodiesel yield from sugarcane molasses, which increased the economic feasibility of the biodiesel production in molasses.

Identifying novel high-yield oleaginous fungal species that produce hydrolytic enzymes is a strategy for reducing the cost of biodiesel production. For example, several strains of *Aspergillus terreus*, an oleaginous fungus that secrets high amounts of lignocellulolytic enzymes, can utilize low-price renewable carbon sources for economical biodiesel production (Khot *et al.*, 2012).

Functional Oils

Many oleaginous microorganisms could produce microbial lipids that are rich in ω-3 and -6 PUFAs such as GLA (C18:3, ω-6), arachidonic acid (ARA, C20:4, ω-6), eicosapentaenoic acid (EPA, C20:5, ω-3), and DHA (C22:6, ω-3). These essential fatty acids (EFAs) that humans cannot synthesize, are now widely accepted by the public for their health benefits (Ward and Singh, 2005). The synthesis pathway of fatty acids in most oleaginous microorganisms is shown in **Fig. 4**. Fatty acids are synthesized from acetyl-CoA and malonyl-CoA by the FAS complex of enzymes. Subsequently, the saturated fatty acid, stearic acid (SA, C18:0) is desaturated and elongated by a battery of reactions and turned into various ω-3/6 PUFAs according to the position of the first double bond from the methyl terminal group (**Fig. 4**) (Ratledge, 2004). Traditionally, the vast majority of EFAs were extracted from cold-water fish and seeds of plants such as evening primrose, borage, and black currant that offer relatively low yields at high costs (Dyal *et al.*, 2005). Also, some concerns remain about using fish oils as nutritional supplements due to the presence of environmental pollutants including dioxins, polychlorinated biphenyl (PCB), and heavy metals that bioaccumulate in fish and become concentrated in the liver and other organs (Ratledge, 2004). Therefore, finding new alternate economical sources and

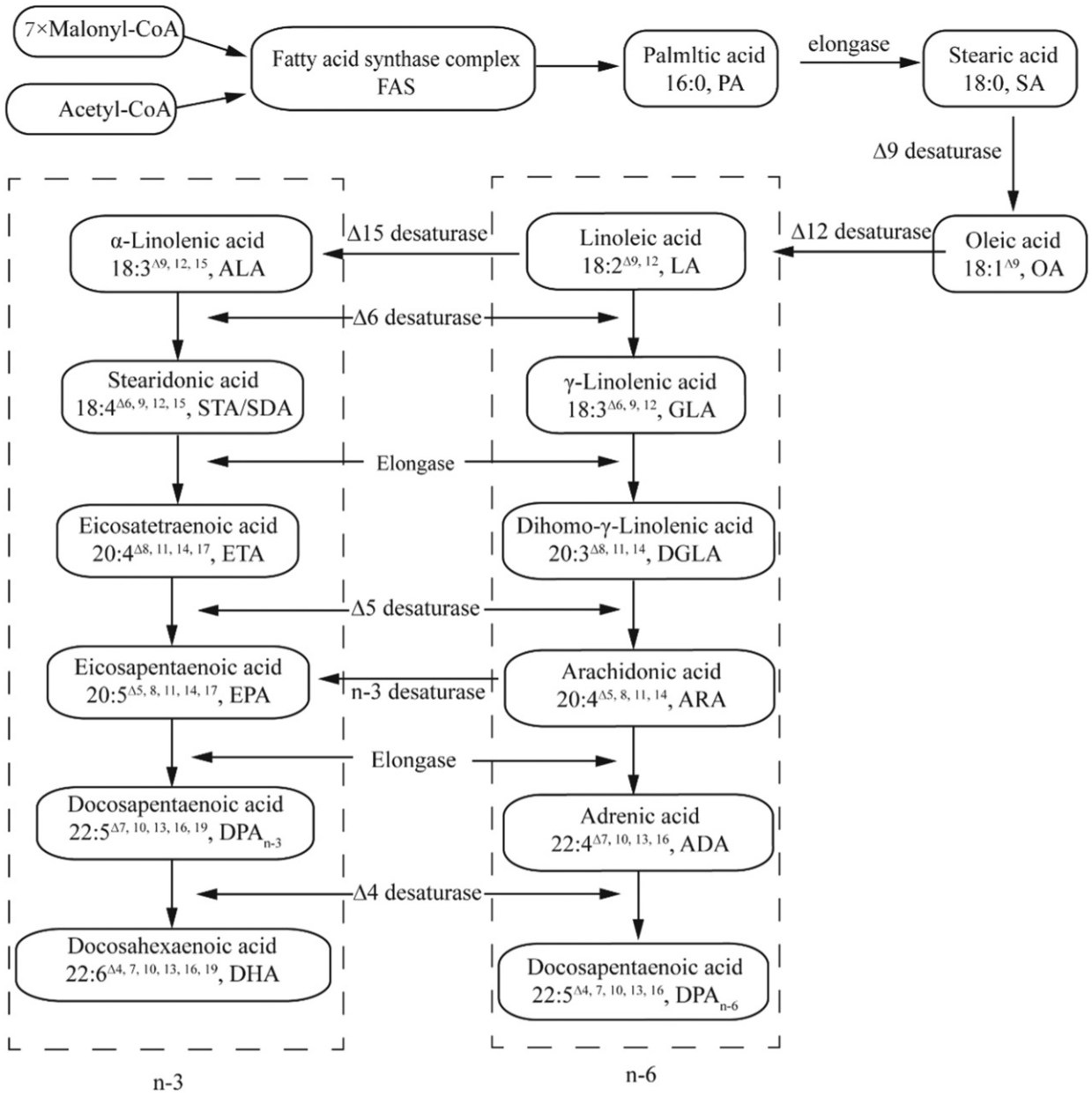

Fig. 4 Biosynthesis of PUFAs in oleaginous microorganisms.

novel dietary products to supplement EFAs has become a hot topic in both academia and industry. With in-depth research on oleaginous fungi, scientists found that fungal species possess unrivaled advantages compared to traditional EFA sources, which make them attractive alternatives for EFAs. Compared to tedious genetic manipulation and strenuous breeding work on plants for EFAs, fungal strains are able to produce a substantial amount of EFAs by controlling growth conditions. Moreover, oleaginous fungi do not require agricultural land for their growth, and can accumulate PUFAs at a level of over 70% of the total cellular lipids. Thus, oleaginous fungi that produce desirable PUFAs (as well as TAGs) have become crucial sources of EFAs (Bellou *et al.*, 2016; Passoth, 2017; Ratledge, 2004). High-value fungal oils will be an important and promising way to provide human diet supplement in the future.

γ -linolenic acid

Among PUFAs, the production of γ -linolenic acid (GLA) by different oleaginous microorganisms, especially fungi, was investigated due to its selective anticancer characteristics and negligible systemic toxicity (Kenny *et al.*, 2000). GLA belongs to the ω-6 PUFAs produced from linoleic acid (LA, C18:2, ω-6) by a Δ-6 desaturase enzyme and it can be further elongated and desaturated to synthesize other long-chain PUFAs including dihomogamma linolenic acid (DGLA, C20:3, ω-6) and ARA. In

industry, GLA could be synthesized and accumulated in different oleaginous fungi such as *U. isabellina* (Papanikolaou *et al.*, 2004), *Cunninghamella echinulate* (Fakas *et al.*, 2007), *U. ramanniana* (Dyal *et al.*, 2005), *M. alpina* (Jang *et al.*, 2005), and *Mucor rouxii* (Mamatha *et al.*, 2008). The filamentous fungus *C. echinulate* produced over 35% of cellular storage lipids and over 11% of GLA under a high C/N ratio of over 100. Following the cellular lipid accumulation, GLA concentration gradually increased in *C. echinulate*, while the highest GLA yield (720 mg L^{-1}, 80 mg g^{-1} of dry biomass) was observed when the C/N ratio reached 163 (Gema *et al.*, 2002). Different nutrient sources for *C. echinulate* ATHUM 4411 varied in GLA yield with 8.7 g L^{-1} of lipids and 1018 mg L^{-1} of GLA from tomato waste hydrolysate and 3.8 g L^{-1} of lipids and 540 mg L^{-1} of GLA from potato starch (Fakas *et al.*, 2008b; Papanikolaou *et al.*, 2007). This suggests the significance of optimal culture conditions for both lipid quality and productivity. *C. echinulate* CCRC 31840 exhibited a higher GLA yield of 964 mg L^{-1} using starch as a substrate, which could be further increased to 1349 mg L^{-1} by optimizing the inoculum (Chen and Liu, 1997; Chen and Chang, 1996).

Many Mucorales species, including *M. circinelloides* and *M. rouxii*, are famous for producing high levels of lipids and GLA (Mamatha *et al.*, 2008). For example, *M. rouxii* CFR-G15 produced a maximum GLA level (18.55%, w/w) using central composite rotatable design (CCRD) along with response surface methodology (RSM) to select optimal medium (Mamatha *et al.*, 2008). *Umbelopsis ramanniana* var. *ramanniana* was reported to produce 13.3% of GLA in the total lipids using 5% dextrose and 1% yeast extract as substrates, which is higher than the GLA yield from evening primrose (Dyal *et al.*, 2005). Similar increases of GLA were observed in *U. isabellina* when grown in a culture broth including 2% octadecanol and 1% yeast extract (Xian *et al.*, 2002), suggesting the enormous potential of oleaginous fungi for producing PUFAs by establishing individual optimum culture conditions.

Arachidonic acid

Arachidonic acid (ARA, C20:4, ω-6) is an important precursor of several key eicosanoid hormones with pharmacologically active metabolites and it has wide applications in medicine, cosmetics, pharmacology, food industry, agriculture, and many other fields (Eroshin *et al.*, 2000). Strains of *Mortierella* species belonging to the subphylum Mortierellomycotina have shown an outstanding capability to produce ARA (Fernandes *et al.*, 2017; Jacobs *et al.*, 2010; Kikukawa *et al.*, 2018). A fast-growing *Mortierella alliacea* YN-15 was shown to accumulate ARA in TAGs: 46.1 g L^{-1} of total dry cell biomass, 19.5 g L^{-1} of total fatty acid with 7.1 g L^{-1} of ARA in 7 d cultivation (Aki *et al.*, 2001). *M. alpina* is well documented as a suitable source for ARA production. *M. alpina* LPM 301 could produce lipids to 16.4% or 18.8% of their dry biomass with 46.0% or 60.4% of ARA content under batch cultivation in glucose-containing media with urea or potassium nitrate as N sources, respectively (Eroshin *et al.*, 2000). *M. alpina* M6 was reported to accumulate 72.3% of ARA in lipids and the yield of ARA could reach 4.82 g L^{-1} (Zhu *et al.*, 2004).

Eicosapentaenoic acid

Eicosapentaenoic acid (EPA, C20:5) is an ω-3 PUFA and has medically therapeutic capabilities against cardiovascular diseases, cancer, schizophrenia, and Alzheimer's disease (Liang *et al.*, 2012; Ursin, 2003). Many fungal species are known to produce a substantial amount of EPA. For example, at low temperature (12°C), *Mortierella* species were able to make EPA comprised 5%–20% of the total extractable fatty acids, including *Mortierella elongata* CBS 121.71 (5%), *M. elongata* IS-5 AKU 3999 (7.9%), *Mortierella hygrophila* IFO 5941 (10.4%), *Mortierella parvispora* 2S-13 AKU 3994 (10.9%), *M. alpina* 2O-17 (17.1%), and *M. alpina* 1–83 (19.8%) (Shimiziu *et al.*, 1988). No obvious EPA was detected in the same strains grown at high temperature (28°C), which produced 18%–48% ARA instead of EPA. Expressing the gene encoding *Saprolegnia diclina* Δ17 desaturase in *M. alpina* ST1358 allowed the transformant to produce EPA at both 12 and 28°C, which had 26.4% EPA of total fatty acid and reached 1.8 g L^{-1} at 28°C (Okuda *et al.*, 2015). The oomycete *Pythium irregulare*, a fungal-like protist that was formerly classified as a fungus, shows great potential in producing EPA. It was reported that *P. irregulare* has the capability to utilize various substrates for its growth and EPA production, including crude soybean oil, soymeal waste, sweet whey permeates, and sucrose waste stream (Cheng *et al.*, 1999; O'Brien *et al.*, 1993). It also has been reported that the oleaginous yeast *Yarrowia lipolytica* was able to produce EPA at 15% of dry cell weight by metabolic engineering approaches (Xie *et al.*, 2015).

Docosahexaenoic acid

Docosahexaenoic acid (DHA, C22:6, ω-3) is a primary structural component of human organs such as the brain and eye. It's essential for the growth and functional development of infant brains and for maintaining normal brain function in adults (Horrocks and Yeo, 1999). The marine fungus *Thraustochytrium aureum* ATCC 34304 has been considered as a promising producer for DHA, which was observed to contain approximately 50% of total fatty acids as DHA, depending on the growth conditions, and produce over 500 mg L^{-1} of DHA under optimized conditions for 6 days (Bajpai *et al.*, 1991). Other *Thraustochytrium* species were reported to offer high amounts of DHA, such as *Thraustochytrium* sp. ATCC 20892 (67.6 mg L^{-1}, 5 days) (Singh *et al.*, 1996) and *Thraustochytrium roseum* ATCC 28210 (1011 mg L^{-1}, 5 days) (Singh and Ward, 1996).

Future Strategies for Productive Oleaginous Fungi

In the past two decades, microbial lipids have been used for food production, and as nutritional supplements, detergent, lubricants, and biodiesel. There are two major barriers to microbial lipid production: the high cost of the glucose substrate and the

low productivity of the oleaginous microorganisms (Lin *et al.*, 2010). At present, many studies have focused on reducing these costs. For large-scale production of SCO, finding suitable feedstock is one key issue for its industrialization (Huang *et al.*, 2017). Various low cost, hydrophilic or hydrophobic substrates were used for SCO production (Huang *et al.*, 2013), and screening of optimal oleaginous fungi has become a vital mission to utilize the low-price substrates. Scientists have been isolating and screening oleaginous strains from field environments, where these fungi could be grown on a large scale. For example, approximately 30 strains of the endophytic fungi were isolated from the oily wood stems of seven oleaginous plants. Fungi belonging to five genera including *Microsphaeropsis*, *Phomopsis*, *Cephalosporium*, *Sclerocystis*, and *Nigrospora*, were able to secrete cellulases and accumulate oils simultaneously, ranging from 21.3% to 35% of dry weight in straw-based solid-state medium (Peng and Chen, 2007).

Aside from screening natural isolates, genetic and metabolic engineering approaches have been carried out to enhance lipid productivity and quality in oleaginous fungi. For example, overexpression of a gene encoding native malic enzyme, a major provider of NADPH for lipid production, led to a 2.5-fold increase in lipid content in the oleaginous fungus *M. circinelloides* (Zhang *et al.*, 2007). In *M. alpina* 1S-4, different approaches such as chemical mutagenesis, RNA interference (RNAi) and overproduction of key enzymes, such as desaturases, were used to engineer fatty acid compositions: the JT-180 mutant lacking Δ12-desaturase had increased Δ5- and Δ6-desaturase activities, resulting in higher levels of Mead acid (MA, C20:3), whereas overexpression of an endogenous Δ12-desaturase encoding gene in the JT-180 strain increased ARA (2.0 g L^{-1}), but reduced MA production (Sakuradani *et al.*, 2002). Disruption of Δ5-desaturase led to substantial production of dihomo-γ-linolenic acid (DGLA, C20:3, ω-6) (Kikukawa *et al.*, 2016), while ARA content could be significantly increased by overexpressing the genes encoding GLELO (Takeno *et al.*, 2005; Wynn and Ratledge, 2000) and multiple desaturases (Δ5, 6, and 12) (Kikukawa *et al.*, 2018). Newer gene-editing technologies such as CRISPR-Cas9 have been used in oleaginous fungi and yeast for genome editing and modification of transcriptional regulation, facilitating more efficient engineering of microbial lipid production (Otoupal *et al.*, 2019; Schwartz and Wheeldon, 2018; Shi *et al.*, 2017).

Conclusions and Perspectives

After decades of research and development, oleaginous fungi have become one of the best performing producers of microbial lipids due to a number of great advantages. Therefore, efforts towards the applications of microbial oils often involve the development of oleaginous fungi. Various biotechnology methodologies, such as mutagenesis, cell fusion, and directed evolution, have been used to engineer fungal strains to improve the productivity of oils and high-value compounds. Additional approaches including genomic, transcriptomic, proteomics, metabolomics, and lipidomic techniques could help to build more impeccable and desirable lipid production, as well as convenient application systems to explore oleaginous fungi. Fungal lipid products could be expanded in the future with better strains and fermentation technologies, which will eventually contribute to meet the demand for energy, food, and essential dietary nutrition of human beings.

References

Ageitos, J.M., Vallejo, J.A., Veiga-Crespo, P., Villa, T.G., 2011. Oily yeasts as oleaginous cell factories. Applied Microbiology and Biotechnology 90, 1219–1227.
Aki, T., Nagahata, Y., Ishihara, K., *et al.*, 2001. Production of arachidonic acid by filamentous fungus, *Mortierella alliacea* strain YN-15. Journal of the American Oil Chemists' Society 78, 599–604.
Alptekin, E., Canakci, M., Sanli, H., 2014. Biodiesel production from vegetable oil and waste animal fats in a pilot plant. Waste Management 34, 2146–2154.
Alvarez, H., Steinbüchel, A., 2002. Triacylglycerols in prokaryotic microorganisms. Applied Microbiology and Biotechnology 60, 367–376.
André, A., Diamantopoulou, P., Philippoussis, A., *et al.*, 2010. Biotechnological conversions of bio-diesel derived waste glycerol into added-value compounds by higher fungi: production of biomass, single cell oil and oxalic acid. Industrial Crops and Products 31, 407–416.
Bagy, M.M.K., Abd-Alla, M.H., Morsy, F.M., Hassan, E.A., 2014. Two stage biodiesel and hydrogen production from molasses by oleaginous fungi and *Clostridium acetobutylicum* ATCC 824. International Journal of Hydrogen Energy 39, 3185–3197.
Bajpai, P.K., Bajpai, P., Ward, O.P., 1991. Optimization of production of docosahexaenoic acid (DHA) by *Thraustochytrium aureum* ATCC 34304. Journal of the American Oil Chemists' Society 68, 509–514.
Bellou, S., Triantaphyllidou, I.-E., Aggeli, D., *et al.*, 2016. Microbial oils as food additives: Recent approaches for improving microbial oil production and its polyunsaturated fatty acid content. Current Opinion in Biotechnology 37, 24–35.
Beopoulos, A., Cescut, J., Haddouche, R., *et al.*, 2009a. *Yarrowia lipolytica* as a model for bio-oil production. Progress in Lipid Research 48, 375–387.
Beopoulos, A., Chardot, T., Nicaud, J.-M., 2009b. *Yarrowia lipolytica*: A model and a tool to understand the mechanisms implicated in lipid accumulation. Biochimie 91, 692–696.
Burton, M., Rose, T.M., Færgeman, N.J., Knudsen, J., 2005. Evolution of the acyl-CoA binding protein (ACBP). Biochemical Journal 392, 299–307.
Carota, E., Crognale, S., D'Annibale, A., Petruccioli, M., 2018. Bioconversion of agro-industrial waste into microbial oils by filamentous fungi. Process Safety and Environmental Protection 117, 143–151.
CharlesDismukes, G., Carrieri, D., Bennette, N., MAnanyev, G., CPosewitz, M., 2008. Aquatic phototrophs: Efficient alternatives to land-based crops for biofuels. Current Opinion in Biotechnology 19, 235–240.
Chatzifragkou, A., Fakas, S., Galiotou-Panayotou, M., *et al.*, 2010. Commercial sugars as substrates for lipid accumulation in *Cunninghamella echinulata* and *Mortierella isabellina* fungi. European Journal of Lipid Science and Technology 112, 1048–1057.
Chatzifragkou, A., Makri, A., Belka, A., *et al.*, 2011. Biotechnological conversions of biodiesel derived waste glycerol by yeast and fungal species. Energy 36, 1097–1108.
Cheirsilp, B., Kitcha, S., 2015. Solid state fermentation by cellulolytic oleaginous fungi for direct conversion of lignocellulosic biomass into lipids: Fed-batch and repeated-batch fermentations. Industrial Crops and Products 66, 73–80.

Chen, H.C., Chang, C.C., 1996. Production of γ-linolenic acid by the fungus *Cunninghamella echinulata* CCRC 31840. Biotechnology Progress 12, 338–341.
Chen, H.-C., Liu, T.-M., 1997. Inoculum effects on the production of γ-linolenic acid by the shake culture of *Cunninghamella echinulata* CCRC 31840. Enzyme and Microbial Technology 21, 137–142.
Cheng, M.H., Walker, T.H., Hulbert, G.J., Raman, D.R., 1999. Fungal production of eicosapentaenoic and arachidonic acids from industrial waste streams and crude soybean oil. Bioresource Technology 67, 101–110.
Daum, G., Wagner, A., Czabany, T., Athenstaedt, K., 2007. Dynamics of neutral lipid storage and mobilization in yeast. Biochimie 89, 243–248.
Demirbas, A., 2007. Progress and recent trends in biofuels. Progress in Energy and Combustion Science 33, 1–18.
Dey, P., Banerjee, J., Maiti, M.K., 2011. Comparative lipid profiling of two endophytic fungal isolates – *Colletotrichum* sp. and *Alternaria* sp. having potential utilities as biodiesel feedstock. Bioresource Technology 102, 5815–5823.
Dourou, M., Aggeli, D., Papanikolaou, S., Aggelis, G., 2018. Critical steps in carbon metabolism affecting lipid accumulation and their regulation in oleaginous microorganisms. Applied Microbiology and Biotechnology 102, 2509–2523.
Du, Z.-Y., Benning, C., 2016. Triacylglycerol accumulation in photosynthetic cells in plants and algae. In: Nakamura, Y., Li-Beisson, Y. (Eds.), Lipids in Plant and Algae Development. Cham: Springer, pp. 179–205.
Durrett, T.P., Benning, C., Ohlrogge, J., 2008. Plant triacylglycerols as feedstocks for the production of biofuels. The Plant Journal 54, 593–607.
Dyal, S.D., Bouzidi, L., Narine, S.S., 2005. Maximizing the production of γ-linolenic acid in *Mortierella ramanniana* var. *ramanniana* as a function of pH, temperature and carbon source, nitrogen source, metal ions and oil supplementation. Food Research International 38, 815–829.
Dyk, J.S.V., Pletschke, B.I., 2012. A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes – Factors affecting enzymes, conversion and synergy. Biotechnology Advances 30, 1458–1480.
Economou, C.N., Aggelis, G., Pavlou, S., Vayenas, D.V., 2011. Single cell oil production from rice hulls hydrolysate. Bioresource Technology 102, 9737–9742.
Economou, C.N., Makri, A., Aggelis, G., Pavlou, S., Vayenas, D.V., 2010. Semi-solid state fermentation of sweet sorghum for the biotechnological production of single cell oil. Bioresource Technology 101, 1385–1388.
Eroshin, V.K., Satroutdinov, A.D., Dedyukhina, E.G., Chistyakova, T.I., 2000. Arachidonic acid production by *Mortierella alpina* with growth-coupled lipid synthesis. Process Biochemistry 35, 1171–1175.
Fakas, S., Čertik, M., Papanikolaou, S., *et al*., 2008a. γ-Linolenic acid production by *Cunninghamella echinulata* growing on complex organic nitrogen sources. Bioresource Technology 99, 5986–5990.
Fakas, S., Papanikolaou, S., Batsos, A., *et al*., 2009b. Evaluating renewable carbon sources as substrates for single cell oil production by *Cunninghamella echinulata* and *Mortierella isabellina*. Biomass and Bioenergy 33, 573–580.
Fakas, S., Galiotou-Panayotou, M., Papanikolaou, S., Komaitis, M., Aggelis, G., 2007. Compositional shifts in lipid fractions during lipid turnover in *Cunninghamella echinulata*. Enzyme and Microbial Technology 40, 1321–1327.
Fakas, S., Papanikolaou, S., Galiotou-Panayotou, M., Komaitis, M., Aggelis, G., 2008b. Organic nitrogen of tomato waste hydrolysate enhances glucose uptake and lipid accumulation in *Cunninghamella echinulata*. Journal of Applied Microbiology 105, 1062–1070.
Fakas, S., Makri, A., Mavromati, M., Tselepi, M., Aggelis, G., 2009a. Fatty acid composition in lipid fractions lengthwise the mycelium of *Mortierella isabellina* and lipid production by solid state fermentation. Bioresource Technology 100, 6118–6120.
Fang, H., Zhao, C., Chen, S., 2016. Single cell oil production by *Mortierella isabellina* from steam exploded corn stover degraded by three-stage enzymatic hydrolysis in the context of on-site enzyme production. Bioresource Technology 216, 988–995.
Felizardo, P., Correia, M.J., Raposo, I., *et al*., 2006. Production of biodiesel from waste frying oils. Waste Management 26, 487–494.
Fernandes, B.S., Messias, M.C.F., de Oliveira Carvalho, P., Zaiat, M., da Cruz Pradella, J.G., 2017. Data of added-value lipid production, arachidonic acid, among other lipids by *Mortierella elongata*, using low cost simulated wastewater. Data in Brief 14, 255–259.
Ferreira, J.A., Lennartsson, P.R., Edebo, L., Taherzadeh, M.J., 2012. Zygomycetes-based biorefinery: Present status and future prospects. Bioresource Technology 135, 523–532.
Gao, D., Zeng, J., Zheng, Y., Yu, X., Chen, S., 2013. Microbial lipid production from xylose by *Mortierella isabellina*. Bioresource Technology 133, 315–321.
Gema, H., Kavadia, A., Dimou, D., *et al*., 2002. Production of γ-linolenic acid by *Cunninghamella echinulata* cultivated on glucose and orange peel. Applied Microbiology and Biotechnology 58, 303–307.
Gupta, V.K., Tuohy, M.G., Ayyachamy, M., Turner, K.M., O'Donovan, A., 2013. Laboratory Protocols in Fungal Biology. New York: Springer.
Hansson, L., Dostálek, M., 1988. Effect of culture conditions on mycelial growth and production of γ-linolenic acid by the fungus *Mortierella ramanniana*. Applied Microbiology and Biotechnology 28, 240–246.
Hao, G., Chen, H., Wang, L., *et al*., 2014. Role of malic enzyme during fatty acid synthesis in the oleaginous fungus *Mortierella alpina*. Applied and environmental microbiology 80, 2672–2678.
Harde, S.M., Wang, Z., Horne, M., Zhu, J.Y., Pan, X., 2016. Microbial lipid production from SPORL-pretreated Douglas fir by *Mortierella isabellina*. Fuel 175, 64–74.
Hill, J., Nelson, E., Tilman, D., Polasky, S., Tiffany, D., 2006. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. Proceedings of the National Academy of Sciences of the United States of America 103, 11206–11210.
Horrocks, L.A., Yeo, Y.K., 1999. Health benefits of docosahexaenoic acid (DHA). Pharmacological Research 40, 211–225.
Huang, C., Chen, X., Xiong, L., *et al*., 2013. Single cell oil production from low-cost substrates: The possibility and potential of its industrialization. Biotechnology Advances 31, 129–139.
Huang, C., Luo, M.T., Chen, X.F., *et al*., 2017. Combined "*de novo*" and "*ex novo*" lipid fermentation in a mix-medium of corncob acid hydrolysate and soybean oil by *Trichosporon dermatis*. Biotechnology for Biofuels 10, 1–11.
Huber, G.W., Corma, A., 2007. Synergies between bio- and oil refineries for the production of fuels from biomass. Angewandte Chemie International Edition 46, 7184–7201.
Jacobs, A., Botha, A., Reddy, J.K., Zyl, W.H.V., 2010. Sunflower press cake as a substrate for eicosapentaenoic acid production by representatives of the genus *Mortierella*. Bioresources 5, 1232–1243.
Jang, H.-D., Lin, Y.-Y., Yang, S.-S., 2005. Effect of culture media and conditions on polyunsaturated fatty acids production by *Mortierella alpina*. Bioresource Technology 96, 1633–1644.
Jin, M., Slininger, P.J., Dien, B.S., *et al*., 2015. Microbial lipid-based lignocellulosic biorefinery: Feasibility and challenges. Trends in Biotechnology 33, 43–54.
Kenny, F.S., Pinder, S., Ellis, I.O., *et al*., 2000. Gamma linolenic acid with tamoxifen as primary therapy in breast cancer. European Journal of Cancer 34, S18–S19.
Khot, M., Kamat, S., Zinjarde, S., *et al*., 2012. Single cell oil of oleaginous fungi from the tropical mangrove wetlands as a potential feedstock for biodiesel. Microbial Cell Factories 11, 1–13.
Khot, M., Katre, G., Zinjarde, S., RaviKumar, A., 2018. Single Cell Oils (SCOs) of oleaginous filamentous fungi as a renewable feedstock: A biodiesel biorefinery approach. In: Kumar, S., Dheeran, P., Taherzadeh, M., Khanal, S. (Eds.), Fungal Biorefineries. Cham: Springer, pp. 145–183.
Kikukawa, H., Sakuradani, E., Ando, A., *et al*., 2016. Microbial production of dihomo-γ-linolenic acid by Δ5-desaturase gene-disruptants of *Mortierella alpina* 1S-4. Journal of Bioscience and Bioengineering 122, 22–26.
Kikukawa, H., Sakuradani, E., Ando, A., Shimizu, S., Ogawa, J., 2018. Arachidonic acid production by the oleaginous fungus *Mortierella alpina* 1S-4: A review. Journal of Advanced Research 11, 15–22.
Kitcha, S., Cheirsilp, B., 2014. Bioconversion of lignocellulosic palm byproducts into enzymes and lipid by newly isolated oleaginous fungi. Biochemical Engineering Journal 88, 95–100.
Knothe, G., 2009. Improving biodiesel fuel properties by modifying fatty ester composition. Energy & Environmental Science 2, 759–766.

Koike, Y., Cai, H., Higashiyama, K., Fujikawa, S., Park, E.Y., 2001. Effect of consumed carbon to nitrogen ratio of mycelial morphology and arachidonic acid production in cultures of *Mortierella alpina*. Journal of Bioscience and Bioengineering 91, 382–389.
Kosa, M., Ragauskas, A.J., 2011. Lipids from heterotrophic microbes: Advances in metabolism research. Trends in Biotechnology 29, 53–61.
Laoteng, K., Certik, M., Cheevadhanark, S., 2011. Mechanisms controlling lipid accumulation and polyunsaturated fatty acid synthesis in oleaginous fungi. Chemical Papers 65, 97–103.
Lewis, T., Nichols, P.D., McMeekin, T.A., 2000. Evaluation of extraction methods for recovery of fatty acids from lipid-producing microheterotrophs. Journal of Microbiological Methods 43, 107–116.
Li, Q., Du, W., Liu, D., 2008. Perspectives of microbial oils for biodiesel production. Applied Microbiology Biotechnology 80, 749–756.
Liang, Y., Zhao, X., Strait, M., Wen, Z., 2012. Use of dry-milling derived thin stillage for producing eicosapentaenoic acid (EPA) by the fungus *Pythium irregulare*. Bioresource Technology 111, 404–409.
Liao, W., Liu, Y., Chen, S., 2007. Studying pellet formation of a filamentous fungus *Rhizopus oryzae* to enhance organic acid production. Applied Biochemistry and Biotechnology 137, 689–701.
Lin, H., Cheng, W., Ding, H.-t., *et al*., 2010. Direct microbial conversion of wheat straw into lipid by a cellulolytic fungus of *Aspergillus oryzae* A-4 in solid-state fermentation. Bioresource Technology 101, 7556–7562.
Madani, M., Enshaeieh, M., Abdoli, A., 2017. Single cell oil and its application for biodiesel production. Process Safety and Environmental Protection 111, 747–756.
Mamatha, S.S., Ravi, R., Venkateswaran, G., 2008. Medium optimization of gamma linolenic acid production in *Mucor rouxii CFR -G15* using RSM. Food and Bioprocess Technology 1, 405–409.
Meeuwse, P., Sanders, J.P.M., Tramper, J., Rinzema, A., 2013. Lipids from yeasts and fungi: Tomorrow's source of biodiesel? Biofuels Bioproducts & Biorefining 7, 512–524.
Meng, X., Yang, J., Xu, X., *et al*., 2009. Biodiesel production from oleaginous microorganisms. Renewable Energy 34, 1–5.
Muhid, F., Nawi, W.N.N.W., Kader, A.J.A., Yusoff, W.M.W., Hamid, A.A., 2008. Effects of metal ion concentrations on lipid and gamma linolenic acid production by *Cunninghamella* sp. 2A1. Online Journal of Biological Sciences 8, 62–67.
Muniraj, I.K., Xiao, L., Hu, Z., Zhan, X., Shi, J., 2013. Microbial lipid production from potato processing wastewater using oleaginous filamentous fungi *Aspergillus oryzae*. Water Research 47, 3477–3483.
Murphy, D.J., 1990. Storage lipid bodies in plants and other organisms. Progress in Lipid Research 29, 299–324.
Navas, M.B., Lick, I.D., Bolla, P.A., Casella, M.L., Ruggera, J.F., 2018. Transesterification of soybean and castor oil with methanol and butanol using heterogeneous basic catalysts to obtain biodiesel. Chemical Engineering Science 187, 444–454.
O'Brien, D.J., Kurantz, M.J., Kwoczak, R., 1993. Production of eicosapentaenoic acid by the filamentous fungus *Pythium irregulare*. Applied and Microbiology Biotechnology 40, 211–214.
Ochsenreither, K., Glück, C., Stressler, T., Fischer, L., Syldatk, C., 2016. Production strategies and applications of microbial single cell oils. Frontiers in Microbiology 7, 1539.
Okuda, T., Ando, A., Negoro, H., *et al*., 2015. Eicosapentaenoic acid (EPA) production by an oleaginous fungus *Mortierella alpina* expressing heterologous the Δ17-desaturase gene under ordinary temperature. European Journal of Lipid Science and Technology 117, 1919–1927.
Otoupal, P., Ito, M., Arkin, A.P., *et al*., 2019. Multiplexed CRISPR-Cas9-based genome editing of *Rhodosporidium toruloides*. mSphere 4, e00099.(19).
Palmqvist, E., Hahn-Hägerdal, B., 2000. Fermentation of lignocellulosic hydrolysates. II: Inhibitors and mechanisms of inhibition. Bioresource Technology 74, 25–33.
Papanikolaou, S., Aggelis, G., 2011. Lipids of oleaginous yeasts. Part I: Biochemistry of single cell oil production. European Journal of Lipid Science and Technology 113, 1031–1051.
Papanikolaou, S., Komaitis, M., Aggelis, G., 2004. Single cell oil (SCO) production by *Mortierella isabellina* grown on high-sugar content media. Bioresource Technology 95, 287–291.
Papanikolaou, S., Chevalot, I., Komaitis, M., Aggelis, G., Marc, I., 2001. Kinetic profile of the cellular lipid composition in an oleaginous *Yarrowia lipolytica* capable of producing a cocoa-butter substitute from industrial fats. Antonie Van Leeuwenhoek 80, 215–224.
Papanikolaou, S., Chevalot, I., Komaitis, M., Marc, I., Aggelis, G., 2002. Single cell oil production by *Yarrowia lipolytica* growing on an industrial derivative of animal fat in batch cultures. Applied Microbiology and Biotechnology 58, 308–312.
Papanikolaou, S., Galiotou-Panayotou, M., Fakas, S., Komaitis, M., Aggelis, G., 2007. Lipid production by oleaginous *Mucorales* cultivated on renewable carbon sources. European Journal of Lipid Science and Technology 109, 1060–1070.
Passoth, V., 2017. Lipids of yeasts and filamentous fungi and their importance for biotechnology. In: Sibirny, A.A. (Ed.), Biotechnology of Yeasts and Filamentous Fungi. Cham: Springer, pp. 149–204.
Peng, X., Chen, H., 2008. Single cell oil production in solid-state fermentation by *Microsphaeropsis* sp. from steam-exploded wheat straw mixed with wheat bran. Bioresource Technology 99, 3885–3889.
Peng, X.-W., Chen, H.-Z., 2007. Microbial oil accumulation and cellulase secretion of the endophytic fungi from oleaginous plants. Annals of Microbiology 57, 239–242.
Predojević, Z.J., 2008. The production of biodiesel from waste frying oils: A comparison of different purification steps. Fuel 87, 3522–3528.
Ratledge, C., 2002. Regulation of lipid accumulation in oleaginous microorganisms. Biochemical Society Transactions 30, 1047–1050.
Ratledge, C., 2004. Fatty acid biosynthesis in microorganisms being used for single cell oil production. Biochimie 86, 807–815.
Ratledge, C., 2014. The role of malic enzyme as the provider of NADPH in oleaginous microorganisms: A reappraisal and unsolved problems. Biotechnology Letters 36, 1557–1568.
Ratledge, C., Wynn, J.P., 2002. The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. In: Laskin, A.I., Bennett, J.W., Gadd, G.M. (Eds.), Advances in Applied Microbiology 51. USA: Elsevier, pp. 1–52.
Ratledge, C., Wynn, J.P., 2020. Oils from microorganisms. In: Shahidi, F. (Ed.), Bailey's Industrial Oil and Fat Products. John Wily & Sons, pp. 1–34.
Reis, C.E.R., Zhang, J., Hu, B., 2014. Lipid accumulation by pelletized culture of *Mucor circinelloides* on corn stover hydrolysate. Applied Biochemistry and Biotechnology 174, 411–423.
Ruan, Z., Zanotti, M., Wang, X., Ducey, C., Liu, Y., 2012. Evaluation of lipid accumulation from lignocellulosic sugars by *Mortierella isabellina* for biodiesel production. Bioresource Technology 110, 198–205.
Ruan, Z., Zanotti, M., Archer, S., Liao, W., Liu, Y., 2014. Oleaginous fungal lipid fermentation on combined acid- and alkali-pretreated corn stover hydrolysate for advanced biofuel production. Bioresource Technology 163, 12–17.
Sajbidor, J., Certík, M., Dobroňová, S., 1988. Influence of different carbon sources on growth, lipid content and fatty acid composition in four strains belonging to mucorales. Biotechnology Letters 10, 347–350.
Sakuradani, E., Kamada, N., Hirano, Y., *et al*., 2002. Production of 5,8,11-eicosatrienoic acid by a Δ5 and Δ6 desaturation activity-enhanced mutant derived from a Δ12 desaturation activity-defective mutant of *Mortierella alpina* 1S-4. Applied Microbiology and Biotechnology 60, 281–287.
Schwartz, C., Wheeldon, I., 2018. CRISPR-Cas9-mediated genome editing and transcriptional control in *Yarrowia lipolytica*. In: Braman, J.C. (Ed.), Synthetic Biology: Methods and Protocols. New York: Springer, pp. 327–345.
Shi, T.-Q., Liu, G.-N., Ji, R.-Y., *et al*., 2017. CRISPR/Cas9-based genome editing of the filamentous fungi: The state of the art. Applied Microbiology and Biotechnology 101, 7435–7443.
Shields-Menard, S.A., Amirsadeghi, M., French, W.T., Boopathy, R., 2018. A review on microbial lipids as a potential biofuel. Bioresource Technology 259, 451–460.
Shimiziu, S., Kawashima, H., Shinmen, Y., Akimoto, K., Yamada, H., 1988. Production of eicosapentaenoic acid by *Mortierella* fungi. Journal of the American Oil Chemists' Society 65, 1455–1459.

Shuib, S., Ibrahim, I., Mackeen, M.M., Ratledge, C., Hamid, A.A., 2018. First evidence for a multienzyme complex of lipid biosynthesis pathway enzymes in *Cunninghamella bainieri*. Scientific Reports 8, 3077.
Singh, A., Ward, O.P., 1996. Production of high yields of docosahexaenoic acid by *Thraustochytrium roseum* ATCC 28210. Journal of Industrial Microbiology 16, 370–d373.
Singh, A., Wilson, S., Ward, O.P., 1996. Docosahexaenoic acid (DHA) production by *Thraustochytrium* sp. ATCC 20892. World Journal of Microbiology and Biotechnology 12, 76–81.
Singhania, R.R., Patel, A.K., Soccol, C.R., Pandey, A., 2009. Recent advances in solid-state fermentation. Biochemical Engineering Journal 44, 13–18.
Soetaert, W., Vandamme, E.J., 2009. Biofuels in perspective. In: Soetaert, W., Vandamme, E.J., (Eds.), Biofuels, pp. 1–8.
Somashekar, D., Venkateshwaran, G., Sambaiah, K., Lokesh, B.R., 2003. Effect of culture conditions on lipid and gamma-linolenic acid production by mucoraceous fungi. Process Biochemistry 38, 1719–1724.
Song, Y., Wynn, J.P., Li, Y., Grantham, D., Ratledge, C., 2001. A pre-genetic study of the isoforms of malic enzyme associated with lipid accumulation in *Mucor circinelloides*. Microbiology 147, 1507–1515.
Srinivasan, S., 2009. The food v. fuel debate: A nuanced view of incentive structures. Renewable Energy 34, 950–954.
Subhash, G.V., Mohan, S.V., 2011. Biodiesel production from isolated oleaginous fungi *Aspergillus* sp. using corncob waste liquor as a substrate. Bioresource Technology 102, 9286–9290.
Subramaniam, R., Dufreche, S., Zappi, M., Bajpai, R., 2010. Microbial lipids from renewable resources: Production and characterization. Journal of Industrial Microbiology & Biotechnology 37, 1271–1287.
Sun, Y., Cheng, J., 2003. Hydrolysis of lignocellulosic materials for ethanol production: A review. Bioresource Technology 83, 1–11.
Takeno, S., Sakuradani, E., Tomi, A., *et al*., 2005. Improvement of the fatty acid composition of an oil-producing filamentous fungus, *Mortierella alpina* 1S-4, through RNA interference with Δ12-desaturase gene expression. Applied and Environmental Microbiology 71, 5124–5128.
Tang, X., Zan, X., Zhao, L., *et al*., 2016. Proteomics analysis of high lipid-producing strain *Mucor circinelloides* WJ11: An explanation for the mechanism of lipid accumulation at the proteomic level. Microbial Cell Factories 15, 35.
Ursin, V., 2003. Modification of plant lipids for human health: Development of functional land-based omega-3 fatty acids. Journal of Nutrition 133, 4271–4274.
Vicente, G., Bautista, L.F., Gutiérrez, F.J., *et al*., 2010. Direct transformation of fungal biomass from submerged cultures into biodiesel. Energy & Fuels 24, 3173–3178.
Wang, J., Wan, W., 2009. Factors influencing fermentative hydrogen production: A review. International Journal of Hydrogen Energy 34, 799–811.
Wang, L., Chen, W., Feng, Y., *et al*., 2011. Genome characterization of the oleaginous fungus *Mortierella alpina*. PIOS One 6, e28319.
Ward, O.P., Singh, A., 2005. Omega-3/6 fatty acids: Alternative sources of production. Process Biochemistry 40, 3627–3652.
Wynn, J.P., Ratledge, C., 2000. Evidence that the rate-limiting step for the biosynthesis of arachidonic acid in *Mortierella alpina* is at the level of the 18:3 to 20:3 elongase. Microbiology 146, 2325–2331.
Xia, C., Wei, W., Hu, B., 2014. Statistical analysis and modeling of pelletized cultivation of *Mucor circinelloides* for microbial lipid accumulation. Applied Biochemistry Biotechnology 172, 3502–3512.
Xia, C., Zhang, J., Zhang, W., Hu, B., 2011. A new cultivation method for microbial oil production: Cell pelletization and lipid accumulation by *Mucor circinelloides*. Biotechnology for Biofuels 4, 15.
Xian, M., Kang, Y., Yan, J., *et al*., 2002. Production of linolenic acid by *Mortierella isabellina* grown on octadecanol. Current Microbiology 44, 141–144.
Xie, D., Jackson, E.N., Zhu, Q., 2015. Sustainable source of omega-3 eicosapentaenoic acid from metabolically engineered *Yarrowia lipolytica*: From fundamental research to commercial production. Applied Microbiology and Biotechnology 99, 1599–1610.
Xu, J., Du, W., Zhao, X., Zhang, G., Liu, D., 2012. Microbial oil production from various carbon sources and its use for biodiesel preparation. Biofuels Bioproducts & Biorefining 7, 65–77.
Zabaruddin, N.H., Mohamed, N.H., Abdullah, L.C., *et al*., 2019. Palm oil-based biodiesel synthesis by radiation-induced kenaf catalyst packed in a continuous flow system. Industrial Crops and Products 136, 102–109.
Zeng, J., Zheng, Y., Yu, X., *et al*., 2013. Lignocellulosic biomass as a carbohydrate source for lipid production by *Mortierella isabellina*. Bioresource Technology 128, 385–391.
Zhang, F., Rodriguez, S., Keasling, J.D., 2011. Metabolic engineering of microbial pathways for advanced biofuels production. Current Opinion in Biotechnology 22, 775–783.
Zhang, H., Zheng, X., Zhang, Z., 2015. The *Magnaporthe grisea* species complex and plant pathogenesis. Molecular Plant Pathology 17, 796–804.
Zhang, J., Hu, B., 2012. Solid-state fermentation of *Mortierella isabellina* for lipid production from soybean hull. Applied Biochemistry and Biotechnology 166, 1034–1046.
Zhang, Y., Adams, I.P., Ratledge, C., 2007. Malic enzyme: The controlling activity for lipid production? Overexpression of malic enzyme in *Mucor circinelloides* leads to a 2.5-fold increase in lipid accumulation. Microbiology 153, 2013–2025.
Zhao, L., Cánovas-Márquez, J.T., Tang, X., *et al*., 2016. Role of malate transporter in lipid accumulation of oleaginous fungus *Mucor circinelloides*. Applied Microbiology and Biotechnology 100, 1297–1305.
Zhao, X., Hu, C., Wu, S., Shen, H., Zhao, Z.K., 2011. Lipid production by *Rhodosporidium toruloides* Y4 using different substrate feeding strategies. Journal of Industrial Microbiology & Biotechnology 38, 627–632.
Zheng, Y., Yu, X., Zeng, J., Chen, H., 2012. Feasibility of filamentous fungi for biofuel production using hydrolysate from dilute sulfuric acid pretreatment of wheat straw. Biotechnology for Biofuels 5, 50.
Zhu, M., Yu, L.J., Liu, Z., Xu, H.B., 2004. Isolating *Mortierella alpina* strains of high yield of arachidonic acid. Letters in Applied Microbiology 39, 332–335.