

Review

Filamentous Fungi for Polyunsaturated Fatty Acid Production: From Strain Resources to Metabolic Engineering

Yajing Yin ^{1,*}, Zhemin Yuan ¹, Meng Zhao ², Weiting Li ¹, Jingqi Wang ¹ and Yueru Zhao ¹

¹ Tianjin Key Laboratory of Food Biotechnology, College of Biotechnology and Food Science, Tianjin University of Commerce, Tianjin 300134, China; 17302247856@163.com (Z.Y.); 18269088395@163.com (W.L.); 18794627008@163.com (J.W.); 16632869596@163.com (Y.Z.)

² Universe Gene Technology (Tianjin) Co., Ltd., Tianjin 300134, China; zhaomeng19921207@163.com

* Correspondence: yinyajing@tjcu.edu.cn; Tel.: +86-022-26667547

Abstract

Polyunsaturated fatty acids (PUFAs) play critical physiological roles in maintaining cardiovascular health, neurodevelopment, and immune regulation, with continuously growing market demand. Traditional supply chains based on fish oil and plant oils face severe challenges including resource depletion, environmental pollution, and sustainability concerns. Single cell oil production via fermentation using filamentous fungi has emerged as a sustainable and economically viable alternative. This review provides a comprehensive overview of research progress on PUFA synthesis by filamentous fungi, covering strain resources, biosynthetic pathways, regulatory mechanisms, major product development, and industrialization prospects. Filamentous fungi have formed a complete spectrum from native high-yielding strains (e.g., *Mortierella alpina*, *Mucor circinelloides*, *Umbelopsis isabellina*, *Thamnidium elegans*, *Cunninghamella echinulata*) to genetically tractable chassis cells (e.g., *Aspergillus oryzae*). In addition, the oleaginous yeast *Yarrowia lipolytica* also plays a non-negligible role in the heterologous synthesis of polyunsaturated fatty acids. Starting from acetyl-CoA, filamentous fungi synthesize saturated fatty acids via the fatty acid synthase system and subsequently convert them to important PUFAs such as arachidonic acid (ARA), γ -linolenic acid (GLA), and eicosapentaenoic acid (EPA) through the sequential catalysis of $\Delta 9$ -, $\Delta 12$ -, $\Delta 6$ -, and $\Delta 5$ -desaturases and elongases. The SNF1 energy sensor, the nitrogen metabolism regulator AreA, and environmental factors constitute a complex multi-layer regulatory network that coordinately modulates fatty acid unsaturation and yield. The 2A peptide-based multigene co-expression platform, cofactor engineering, competitive pathway blockade, and fermentation process optimization have synergistically enabled the targeted accumulation of specific PUFAs. Nevertheless, industrialization of filamentous fungal PUFA production still faces major bottlenecks. We also provide a perspective on the industrial potential of filamentous fungi as “lipid cell factories” for the production of polyunsaturated fatty acids (PUFAs). This review aims to provide a comprehensive knowledge framework for researchers in related fields and to inform future fundamental research and industrial translation.

Keywords: filamentous fungi; polyunsaturated fatty acids; *Mortierella alpina*; metabolic engineering; fermentation optimization

Academic Editor: George N. Bennett

Received: 6 August 2026

Revised: 29 September 2026

Accepted: 29 September 2026

Published: 30 September 2026

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1. Introduction

Polyunsaturated fatty acids (PUFAs) are a class of fatty acids containing two or more carbon-carbon double bonds in their hydrocarbon chains. Based on the position of the first double bond, PUFAs are classified into the ω -3 series, including α -linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), and the ω -6 series, including linoleic acid (LA), arachidonic acid (ARA), and γ -linolenic acid (GLA) [1]. Because humans and most higher animals lack the desaturases required to introduce double bonds at the n-3 and n-6 positions of fatty acid chains, these PUFAs cannot be synthesized de novo and must be obtained through the diet, thus being designated essential fatty acids [2]. PUFAs perform irreplaceable biological functions in maintaining human health. ω -3 PUFAs reduce the risk of cardiovascular diseases, DHA plays a critical role in infant neurodevelopment and visual function, and PUFAs and their derivatives, such as eicosanoids, participate in regulating inflammatory responses and cell signal transduction. Recent studies have also revealed potential roles for ω -3 PUFAs in preventing diabetes, cognitive decline, depression, and certain cancers [3,4]. Recent studies have also revealed that GLA produced by the filamentous fungus *Cunninghamella elegans* using agro-industrial wastes exhibits immunomodulatory and anti-inflammatory activities, providing an environmentally friendly microbial platform for industrial applications [5]. Owing to these extensive health benefits, PUFAs have become important raw materials in functional foods, dietary supplements, and pharmaceuticals [2,3].

Traditional sources of PUFAs are mainly marine fish and their processing byproducts, as well as plant oils, which suffer from critical drawbacks including resource unsustainability, unstable yield and quality, environmental pollution, low bioconversion efficiency, and poor chemical stability [6,7]. Microbial production of PUFAs has emerged as a highly promising alternative, offering multiple advantages: short production cycles, independence from geographic and climatic constraints, high purity of microbial oils with no risk of environmental contaminant accumulation, efficient conversion of inexpensive agricultural and industrial byproducts in line with green and sustainable development principles, and the ability to tailor specific PUFA profiles through strain screening, metabolic engineering, and fermentation optimization [8–10]. Among PUFA-producing microorganisms, filamentous fungi have attracted particular attention due to their unique physiological and metabolic characteristics. Among them, *Mortierella alpina* is the most extensively studied species and has been industrially applied to ARA production [11]. *Mucor circinelloides* has drawn interest for its efficient GLA synthesis and accumulation [12], and *C. elegans* has also been reported to possess substantial GLA content [13,14]. Moreover, many oleaginous Zygomycetes possess inherent advantages in lipid accumulation and polyunsaturated fatty acid production, such as *Umbelopsis isabellina* and *Thamnidium elegans* [15,16]. Beyond native high-yielding strains, genetically tractable filamentous fungi such as *Aspergillus oryzae* can serve as platforms for constructing efficient PUFA production systems [17]. Thus, filamentous fungi occupy an important position not only in native PUFA production but also in synthetic biology-based strain improvement.

In recent years, advances in genomics, metabolomics, proteomics, and CRISPR gene editing tools have propelled substantial progress in PUFA synthesis by filamentous fungi. However, a review that comprehensively integrates the entire research chain from “strain resources” to “molecular mechanisms” to “engineering applications” for PUFA synthesis in filamentous fungi is still lacking. This review adopts a dual-layer structure of “common principles plus specific applications” to provide a comprehensive summary of research progress on PUFA synthesis by filamentous fungi. It first summarizes the strain resources and their characteristics, then elucidates the biosynthetic pathways and key enzymes, analyzes transcriptional, and metabolic flux regulatory mechanisms, and finally reviews progress in metabolic engineering and fermentation technology for major PUFA products.

Through comprehensive integration and critical analysis, this review aims to provide researchers in this field with a comprehensive knowledge map and to inform future fundamental research and industrial applications.

2. Filamentous Fungi for PUFA Production

2.1. Mortierellaceae: The Core Family for ARA Production

The family Mortierellaceae is the most representative group of filamentous fungi for PUFA synthesis, encompassing multiple species with significant industrial value. Among these, *M. alpina* is the most extensively studied and widely applied.

2.1.1. *Mortierella alpina*

The *M. alpina* 1S-4 strain is a model organism in this field, capable of accumulating large quantities of triacylglycerols rich in C20 PUFAs (Table 1). Its triacylglycerol yield can reach 20 g/L in culture medium, with ARA (C20:4 ω -6) constituting 30–70% of total fatty acids [18]. ARA-rich oil from *M. alpina* has received multiple “no questions” letters from the U.S. FDA in response to GRAS notices (GRN 41, 80, 94, 326, 730, 963, and 1259). Documentation states that since the early 1990s, fungal oil as a source of ARA has been used in commercially available infant formula in at least 50 countries. The EU has authorized ARA-rich oil from *M. alpina* as a novel food ingredient for use in infant formula, follow-on formula, and formulae for premature infants, and it has subsequently been included in the EU Union list of novel foods (EU 2017/2470) [11]. Research on *M. alpina* has established a comprehensive genetic manipulation system. Through mutagenesis breeding, gene functional analysis, and molecular breeding, the functions of PUFA biosynthesis-related enzymes have been elucidated, and engineered strains capable of producing specific PUFAs (including ARA) have been successfully constructed [18,19]. In recent years, research focus has shifted toward enhancing fatty acid precursor supply and NADPH levels to improve lipid yield and fatty acid unsaturation, as well as optimizing PUFA synthesis pathways through genetic modification to improve fatty acid profiles. Multi-omics approaches have been applied to reveal the global regulatory mechanisms of lipid synthesis in *M. alpina*. However, its filamentous, multinucleate mycelial structure makes gene transformation, homologous recombination, and monoclonal isolation exceptionally difficult. Moreover, its long growth cycle and insufficient carbon-to-lipid conversion efficiency pose challenges for process scale-up [11].

2.1.2. Genus *Linnemannia*

Beyond *M. alpina*, several Mortierellaceae species capable of PUFA production have attracted considerable attention, including *Linnemannia elongata* (formerly *M. elongata*), *L. hyalina* (formerly *M. hyalina*) (Table 1) [16]. Species in the genus *Linnemannia* contain C20 PUFAs and are potential producers of long-chain PUFAs such as ARA and EPA. The major PUFA in *L. elongata* and *L. hyalina* is ARA, accounting for 14.3–22.8% and 11.3–14.4% of total fatty acids, respectively [20].

2.2. Mucoromycota: Important Sources of GLA and Diverse PUFAs

Fungi of the order Mucorales represent another important group of PUFA-producing filamentous fungi, particularly advantageous for GLA (C18:3 ω -6) production.

2.2.1. *Mucor circinelloides*

M. circinelloides is a dimorphic fungus capable of growing as either unicellular yeast-like cells or filamentous hyphae depending on oxygen availability, and it is a widely used model microorganism for studying lipid accumulation mechanisms (Table 1) [21]. Since

the 1980s, researchers have systematically studied *M. circinelloides*, which was the first strain used for industrial microbial oil production [22]. In 2007, the USA Department of Energy Joint Genome Institute sequenced the genome of *M. circinelloides* CBS 277.49, although its lipid content was only 15% [23]. In 2015, Tang et al. [24] isolated a high-lipid strain, *M. circinelloides* WJ11, with lipid content reaching 36%, and completed its whole-genome sequencing. Subsequently, comparative genomics [25], proteomics [26], metabolomics [27], and biochemical activity analyses [24] between the low-lipid strain CBS 277.49 and the high-lipid strain WJ11 elucidated the lipid accumulation mechanisms in *M. circinelloides* WJ11. Metabolic engineering of *M. circinelloides* WJ11 represents a promising approach for enhancing GLA production.

2.2.2. *Cunninghamella echinulata*

C. echinulata is another filamentous fungus with GLA production potential (Table 1). Its GLA yield is significantly influenced by the carbon source: when trehalose is used as the carbon source, the *C. echinulata* FR3 strain achieves GLA content of 21% of total lipids, substantially higher than the 13% observed under glucose cultivation. Transcriptomic and lipidomic analyses revealed that trehalose upregulates $\Delta 6$ -desaturase expression, thereby increasing the GLA proportion. The elevated GLA is exported to the extracellular environment through active phosphatidylinositol (PI) ion channels, reducing intracellular accumulation. Trehalose may also form trehalose-PUFA complexes that prevent GLA peroxidation [13]. Additionally, *C. echinulata* can utilize agricultural and industrial wastes such as corn steep liquor and whey concentrate as nitrogen sources for GLA-rich microbial oil production, with GLA concentrations reaching 800 mg/L when cultured on tomato waste hydrolysate [14]. Under cultivation with glucose at an initial substrate concentration of 60 g/L, *C. echinulata* produced up to 3.9 g/L of lipids, with GLA reaching 19.5%. It also exhibited good growth characteristics on molasses-based medium, producing significant amounts of SCO and GLA, accompanied by pronounced substrate decolorization [28].

2.2.3. Genus *Umbelopsis*

Species of the genus *Umbelopsis* have also been found to produce PUFAs, including *Umbelopsis ramanniana* (formerly *M. ramanniana*), *U. isabellina* (formerly *M. isabellina*), and *U. vinacea* (formerly *M. vinacea*) (Table 1) [16]. Species in the genus *Umbelopsis* primarily produce C18 PUFAs such as GLA and LA. The major PUFA in *U. ramanniana*, *U. isabellina*, and *U. vinacea* is GLA, accounting for 13.2–31.4%, 6.9–22.9%, and 22.0% of total fatty acids, respectively [20]. Fatty acid profiles vary significantly among strains within species, making strain screening the first step in industrial development. Temperature, pH, carbon and nitrogen sources, metal ions, and medium supplements all significantly influence fatty acid composition, and multi-parameter optimization can achieve targeted enrichment of desired PUFAs [29]. For example, one study reported that *U. isabellina* ATHUM 2935 produced 8.5 g/L of lipids, corresponding to 83.3% (*w/w*) of DCW, under glucose-based, nitrogen-limited conditions, with GLA accounting for 3.4% (*w/w*) of total fatty acids and a lipid conversion yield of approximately 0.25 g/g glucose consumed. This represents one of the highest lipid contents ever reported for wild-type strains [30]. The same strain has also been reported to reach a DCW of 35.9 g/L with a lipid content of 50–55% (*w/w*) in nitrogen-limited media with high initial sugar concentrations (e.g., 100 g/L) [31].

2.2.4. *Thamnidium elegans*

Papanikolaou et al. (2010) cultivated *T. elegans* CCF-1465 in submerged shake-flask cultures using low-cost sugars (glucose, fructose, and sucrose) as carbon sources. They found that lipid production increased significantly with increasing sugar concentration in

the medium, regardless of the individual sugar used. Maximum lipid production exceeded 9 g/L, with lipids accounting for 70% (*w/w*) of dry cell weight and a conversion yield of more than 20% (*w/w*) lipid per unit of sugar consumed. The type and concentration of sugar, as well as fermentation time, appeared to affect the distribution of fatty acids in the products; overall, GLA accounted for approximately 5.5–12.3% (*w/w*) of total fatty acids, and a maximum production of about 510 mg/L was obtained with sucrose at an initial concentration of 80 g/L as the carbon source [15]. In addition, when glycerol was used as the carbon source, 11.6 g/L of oil was produced, with lipids accounting for 71.1% (*w/w*) of dry cell weight and a maximum GLA concentration of 371 mg/L [32].

2.3. Other High-Potential PUFA-Producing Strains

Other oleaginous fungi have also been identified within the Entomophthorales, including *Neoconidiobolus nanodes* (formerly *Conidiobolus nanodes*) and *Entomophthora exitalis*, which produce 25% oil containing both ω -3 and ω -6 PUFAs, with ARA at 16% and 18%, respectively. Furthermore, the fungus-like Oomycota harbor PUFA-rich potential strain resources. *Pythium irregulare*, an oomycete, possesses both ω -3 and ω -6 synthesis enzyme systems, including Δ 6-desaturase PiD6 and Δ 5-desaturase PiD5, enabling simultaneous production of EPA and ARA, making it a promising candidate for co-production of these two PUFAs [33–36]. Recent studies have also demonstrated that *P. irregulare* can utilize food industry by-products and wastes for fermentative EPA production, achieving a final concentration of up to 155 mg/L [37]. *Saprolegnia parasitica*, belonging to the Oomycota genus *Saprolegnia*, produces 10% oil containing ARA and EPA at 19% and 18%, respectively [38].

2.4. Genetically Tractable Filamentous Fungal Chassis Cells

Although some filamentous fungi have limited native PUFA synthesis capacity, their well-established genetic manipulation systems make them ideal chassis cells for heterologous expression of PUFA synthesis genes.

2.4.1. *Aspergillus oryzae*

A. oryzae has a long history of application in food fermentation and an excellent safety record, being generally recognized as safe (GRAS) by the USA FDA and internationally regarded as edible [39,40]. This strain produces relatively few secondary metabolites [41,42], making it an ideal host for heterologous production of high-value secondary metabolites [43–45]. Researchers introduced Δ 6-desaturase and Δ 6-elongase genes from *M. alpina* into an *A. oryzae* *faaA* mutant through heterologous expression, successfully producing free dihomog- γ -linolenic acid (DGLA) (Table 1) [42]. Through a series of subsequent modifications, the DGLA titer reached 533 mg [17,46,47]. Additionally, researchers have investigated the production of high-value fatty acids in esterified fatty acid (EFA) form and yield improvement through metabolic modification. They first heterologously introduced Δ 6-desaturase and Δ 6-elongase genes from *Pythium* sp. into *A. oryzae* BCC7051, successfully obtaining EFA-form DGLA [48]. Subsequent metabolic engineering further improved the yield of esterified DGLA [49].

2.4.2. *Aspergillus nidulans*

A. nidulans is a classical model organism for fungal genetics, possessing abundant genetic tools and a well-characterized genomic background [50]. Although its native PUFA yield is low, it can serve as a heterologous expression platform for PUFA production research. Recent successful application of fungal artificial chromosome (FAC) technology in *A. nidulans* has further expanded its potential as a heterologous expression platform (Table 1) [51,52].

2.5. Oleaginous Yeast Chassis: *Yarrowia lipolytica*

In addition, the oleaginous yeast *Y. lipolytica* plays a non-negligible role in the heterologous synthesis of polyunsaturated fatty acids (PUFAs). It should be noted that *Y. lipolytica* is a non-conventional dimorphic oleaginous yeast belonging to the subphylum Saccharomycotina within the phylum Ascomycota (NCBI Taxonomy ID: 4952). Its typical growth form is unicellular yeast, rather than filamentous fungi that grow primarily as mycelia. However, *Y. lipolytica* possesses excellent intrinsic lipid synthesis capacity, well-established genetic manipulation tools, and favorable industrial fermentation characteristics, such as the ability to utilize diverse inexpensive carbon sources, tolerate high metabolic loads of acetyl-CoA, and maintain stable cell growth under industrial conditions. It has therefore been widely used for the production of rare PUFAs, including ω -3 EPA, DHA, and ALA, as well as ω -6 ARA and GLA [53].

In EPA production, the high-performance strain Z5567 obtained by DuPont through a series of genetic modifications harbors 41 copies of 19 different genes; its EPA production reaches approximately 50% of fatty acid methyl esters (FAMES), with a total lipid content of >50% DCW and EPA at approximately 25% DCW [9]. Another study reported an engineered *Y. lipolytica* strain that synthesizes DHA using a PKS-like PUFA synthase from myxobacteria. In the resulting transformants, DHA reached up to 16.8% of TFAs, and after fermentation process optimization, the DHA titer reached 350 mg/L [54]. A separate study used a bifunctional $\Delta 12/\Delta 15$ desaturase combined with low-temperature fermentation to achieve ALA accumulation of nearly 1.4 g/L [55].

In addition, *Y. lipolytica* has been engineered to produce ω -6 PUFAs such as GLA and ARA. Chuang et al. (2010) co-expressed the $\Delta 6$ and $\Delta 12$ bifunctional desaturase genes from *M. alpina* in *Y. lipolytica*, enabling the recombinant strain to synthesize GLA from endogenous oleic acid (OA) and LA, with GLA accounting for 20% of TFAs [56]. Sun et al. (2017) overexpressed a codon-optimized $\Delta 6$ -desaturase from *M. alpina* and employed a temperature-shift strategy (cultivation at 28 °C for 1 day followed by 20 °C for 6 days), ultimately achieving a GLA titer of 71.6 mg/L in a bioreactor [57]. Liu et al. (2019) reconstructed the Δ -9 pathway from *Isochrysis galbana* in *Y. lipolytica* through pathway engineering, achieving an ARA titer of 91.5 mg/L. To further increase the ARA titer, the researchers designed a novel enzyme fusion strategy combining a Δ -9 elongase and a Δ -8 desaturase, with combinatorial optimization of different linker peptides. Ultimately, using the reconstructed strain RH-4 containing a rigid linker (GGGGS), combined with pathway engineering and synthetic enzyme fusion, the ARA titer was increased by 29% to 118.1 mg/L [58]. Wang et al. (2023) systematically explored the optimal biosynthetic route for ω -6 PUFAs in *Y. lipolytica* by comparing the $\Delta 6$ pathway from *M. alpina* with the $\Delta 8$ pathway from *Isochrysis galbana*. Through strategies such as enhancing the supply of fatty acid biosynthetic precursors, increasing carriers for fatty acid desaturation, and preventing fatty acid degradation, the proportion of ω -6 PUFAs in total fatty acids was effectively increased. Finally, the customized strain produced GLA, DGLA, and ARA at 22.58%, 46.65%, and 11.30% of TFAs, respectively, with corresponding shake-flask titers of 386.59, 832.00, and 191.76 mg/L. Notably, the DGLA proportion of 46.65% represents the highest level reported in *Y. lipolytica* [59]. Together, these studies demonstrate that *Y. lipolytica*, as an oleaginous yeast chassis, has exhibited significant production potential for the heterologous synthesis of rare PUFAs, complementing filamentous fungal platforms.

2.6. Discovery and High-Throughput Screening of Novel Strain Resources

Beyond the well-studied species described above, isolating and screening novel PUFA-producing filamentous fungi from diverse ecological niches is becoming an important avenue for expanding strain resources.

Fungi of the phylum Mucoromycota are recognized as good sources of PUFAs and promising candidates for lipid production using low-value substrates. The establishment of high-throughput screening methods has greatly accelerated this process. For example, the Duetz microtiter plate system combined with Fourier-transform infrared spectroscopy (FTIR) has been shown to enable reproducible high-throughput screening of oleaginous microorganisms and has successfully identified multiple strains with high-value ω -6 PUFA production potential [60].

Extreme environments also represent important sources of novel strains. Psychrotolerant strains isolated from low-temperature environments, such as *Mucor racemosus* AH1 from Egyptian fecal samples, can efficiently produce PUFAs at 10–20 °C [61]. Three fungal strains isolated from Antarctic environmental matrices with high PUFA proportions showed increased linolenic acid (C18:3) production when grown at 10 °C [62]. Soil ecosystems also harbor abundant oleaginous fungal resources—12 fungal strains isolated from soil all accumulated more than 20% lipid on a dry cell weight basis, with one identified as *Gongronella butleri* HMA-10 achieving a maximum biomass of 15.8 ± 0.8 g/L and lipid content of $40.6 \pm 1.7\%$ [62]. Tropical lakes have also been reported as novel sources of oleaginous fungi, with 46.15% of isolates showing lipid yields exceeding 20% of dry biomass [63]. These newly isolated strains not only enrich the strain diversity for PUFA production but also provide new biological resources for developing more sustainable fermentation processes, such as those using waste streams as substrates.

Table 1. Overview of major filamentous fungal strain resources for PUFA production.

Microorganism	Main PUFA Products	Lipid Content (%) DCW	Major PUFA Content (%) TFA	Titer	Culture Conditions	Genetic Tool Availability	Industrialization Status	Key References
<i>Mortierella alpina</i> 1S-4	ARA	50–60%	30–70%	TAG 20 g/L; ARA 3.0 g/L	Glucose, 10 L fermenter	Well-established	Commercialized	[18]
<i>Linnemannia elongata</i>	ARA	Similar to <i>M. alpina</i>	14.3–22.8%	—	Glucose, shake-flask	Limited	Laboratory-scale	[29]
<i>Umbelopsis ramanniana</i>	GLA	24%	9–13%	GLA 430 mg/L	glycerol, shake-flask	Limited	Laboratory-scale	[64]
<i>Umbelopsis isabelina</i>	GLA	83.3%	3.4%	Lipids 8.9 g/L	Glucose, 3 L bioreactor	Limited	Laboratory-scale	[30]
<i>Mucor circinelloides</i> WJ11	GLA	36%	14.5%	Lipids 4.1 g/L	Xylose, shake-flask	Well-established	Laboratory-scale	[24]
<i>Cunninghamella echinulata</i>	GLA	25.11%	21%	GLA 0.52 g/L	Trehalose, shake-flask	Limited	Laboratory-scale	[13]
<i>Cunninghamella elegans</i>	GLA	30.54%	10.77%	GLA 668 mg/L; PUFA 1906 mg/L; biomass 20.3 g/L	stalk hydrolysates, nitrogen-excess, shake-flask	Limited	Laboratory-scale	[65]
<i>Cunninghamella blakesleeana</i> -JSK2	GLA	41%	21%	biomass 5.0 g/L; GLA 430 mg/L	Glucose, shake-flask	Limited	Laboratory-scale	[66]
<i>Thamnidium elegans</i>	GLA	47.1%	6.76%	GLA 1014 mg/L	Glucose, shake-flask	Limited	Laboratory-scale	[67]
<i>Aspergillus oryzae</i>	Heterologous expression	—	—	DGLA 533 mg/L	Glucose, shake-flask	Well-established	Heterologous platform	[17]
<i>Aspergillus nidulans</i>	Heterologous expression	—	—	—	—	Well-established	Research platform	[50]

DCW: dry cell weight; TFA: total fatty acids; titer: fatty acid volumetric concentration; laboratory-scale: shake-flask or small bioreactor studies only; commercialized: products currently on the market or with regulatory approval. “—” indicates data not reported.

3.1. The De Novo Fatty Acid Synthesis Pathway

The de novo fatty acid synthesis pathway in filamentous fungi is evolutionarily conserved. This pathway comprises three key stages: acetyl-CoA generation, malonyl-CoA synthesis, and chain elongation with modification.

3.1.1. Acetyl-CoA Supply

Acetyl-CoA is the primary starting substrate for fatty acid synthesis. In oleaginous fungi, sufficient acetyl-CoA supply is a prerequisite for efficient lipid accumulation. In eukaryotes, the mitochondrial pyruvate dehydrogenase (PDH) complex irreversibly decarboxylates the glycolytic product pyruvate to generate acetyl-CoA in the mitochondria, serving as the rate-limiting enzyme in this reaction [73–75]. Subsequently, acetyl-CoA and pyruvate are sequentially converted to citrate by citrate synthase (CS), and citrate is then transported to the cytosol by citrate transporter (CT) or the mitochondrial citrate- α -ketoglutarate transporter (YHM), where ATP: citrate lyase (ACL) cleaves it to regenerate acetyl-CoA. Alternatively, acetyl-CoA can be generated from acetate in the cytosol by acetyl-CoA synthetase (ACS) or released from fatty acid and amino acid oxidation in mitochondria [76]. Acetyl-CoA carboxylase (ACC) catalyzes the conversion of acetyl-CoA to malonyl-CoA, which is another rate-limiting step in fatty acid synthesis [76].

3.1.2. Fatty Acid Synthase (FAS) System

Fatty acid synthesis in fungi is primarily catalyzed by the type-I fatty acid synthase (type-I FAS) system. FAS is a multi-domain enzyme complex composed of eight different catalytic domains that utilizes acetyl-CoA and malonyl-CoA as substrates and NADPH as the reducing power to progressively elongate the carbon chain, ultimately generating C16:0 (palmitic acid) or C18:0 (stearic acid) saturated fatty acids [77–79]. Following de novo synthesis, C16:0 and C18:0 saturated fatty acids serve as substrates for subsequent desaturation and elongation. In the oleaginous fungus *M. alpina*, FAS activity is closely correlated with lipid accumulation capacity [80]. Recent comparative genomic studies have identified two novel types of fatty acid synthases in the Mortierellaceae, providing new perspectives on the molecular basis of the high oleagenicity of this family [81].

3.2. PUFA Desaturation and Elongation Pathways

In oleaginous microorganisms such as Mortierellaceae species, the PUFA synthesis pathway includes one fatty acid Δ 5-desaturase (FADS5), one Δ 12-desaturase (FADS12), one ω -3 desaturase (FADS15), two genes encoding Δ 6-desaturase (FADS6), two Δ 9-desaturases (FADS9), and three fatty acid elongases (ELO). Unsaturated fatty acid synthesis in *M. alpina* begins with the introduction of a double bond into stearic acid (C18:0) to generate the monounsaturated fatty acid oleic acid (OA, C18:1), a reaction catalyzed by FADS9. FADS9 plays a key role in maintaining the balance between saturated and unsaturated fatty acids and provides substrates for subsequent desaturation and elongation pathways [82].

3.2.1. ω -6 Series PUFA Synthesis

ω -6 series PUFA synthesis begins with OA. First, Δ 12-desaturase introduces a double bond at the Δ 12 position of oleic acid to generate LA, a key intermediate in ω -6 series PUFA synthesis. Subsequently, Δ 6-desaturase (FADS6) introduces a double bond at the Δ 6 position of LA to generate GLA, which itself is a biologically active PUFA and a precursor for long-chain PUFA synthesis. Next, the elongase GLELO (also known as C18- Δ 6-polyunsaturated fatty acyl-CoA elongase) catalyzes the elongation of GLA, adding two carbon atoms at the carboxyl end to generate DGLA. Finally, Δ 5-desaturase introduces a

double bond at the $\Delta 5$ position of DGLA to generate ARA. ARA is one of the most important long-chain PUFAs in the ω -6 series and the primary target product of industrial *M. alpina* fermentation [11].

3.2.2. ω -3 Series PUFA Synthesis

ω -3 series PUFA synthesis proceeds in parallel with the ω -6 pathway. ALA is generated from LA by $\Delta 15$ -desaturase (FADS15, also known as $\omega 3$ -desaturase). ALA then sequentially undergoes $\Delta 6$ -desaturation, GLELO-catalyzed elongation, and $\Delta 5$ -desaturation to generate stearidonic acid (SDA, 18:4 $\Delta 6,9,12,15$), eicosatetraenoic acid (ETA, 20:4 $\Delta 8,11,14,17$), and EPA, respectively. As the most important ω -3 desaturase in *M. alpina*, FADS15 converts ω -6 PUFAs to ω -3 PUFAs [11]. Notably, the native EPA yield in *M. alpina* is low, and efficient EPA production is primarily achieved through genetic engineering introducing heterologous $\omega 3$ -desaturase or $\Delta 17$ -desaturase genes [83].

3.3. Cofactor Requirements and Metabolic Supply

PUFA synthesis depends not only on enzyme catalysis but also critically on cofactors, particularly an adequate supply of NADPH. NADPH serves as the reducing power in fatty acid synthesis, with each two-carbon elongation consuming two molecules of NADPH and each double bond introduction also consuming NADPH. Therefore, the NADPH supply capacity directly limits the rate and yield of PUFA synthesis. In *M. alpina*, the pentose phosphate pathway is the primary contributor of NADPH for fatty acid synthesis, with glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) playing a key role in NADPH generation [84–86]. Beyond this, NADPH can also be generated through multiple other pathways. For example, malic enzyme (ME) is considered an important source of NADPH in oleaginous microorganisms, with its activity closely correlated with lipid accumulation levels. Additionally, the glutamate metabolism pathway can provide NADPH for de novo fatty acid synthesis [87–89]. The tricarboxylic acid cycle is also an important source of NADH in mitochondria and NADPH in the cytosol [87]. Understanding the relative contributions of different NADPH supply pathways is of great significance for improving PUFA yields through metabolic engineering.

3.4. Ex Novo Lipid Accumulation and Biomodification of Fatty Substrates

Oleaginous microorganisms can accumulate lipids through two biochemical processes: the “de novo” pathway, which uses hydrophilic substrates such as sugars and related compounds (e.g., glucose, glycerol) as carbon sources; and the “ex novo” pathway, which uses hydrophobic substrates such as oils, fats, and fatty acids. In the ex novo process, fatty acids in the medium are incorporated at different rates and serve as “substrates” for intracellular biotransformation, being modified and subsequently stored in the form of triacylglycerols [90]. The main genera employed in ex novo lipid production include *Mucor*, *Mortierella*, *Thamnidium*, *Cunninghamella*, and *Rhizopus*. These fungi possess active hydrolytic and modifying enzyme systems (e.g., lipases, desaturases, elongases), enabling them to efficiently utilize hydrophobic substrates [91].

The ex novo process may generate “new” fatty acid profiles that do not exist in the substrate, thereby converting low-value fatty materials into high-value oils through biomodification. When fungi are cultivated on fatty substrates (e.g., sunflower oil, olive oil, animal fats), in some strains they not only accumulate exogenous fatty acids but also synthesize new fatty acids that were originally absent from the substrate through desaturation and elongation reactions [90]. For example, *M. alpina* CCF-185 can synthesize 465 mg/L of ARA on sunflower oil medium [92]; *C. echinulata* CCF-103 can produce 373 mg/L of GLA under the same conditions. However, it should be noted that the activities of

desaturase and elongase systems are strain-specific and may be inhibited by exogenous fatty acids in certain fungi [93].

When fats are used as co-substrates with glucose, the order of carbon source utilization varies depending on the strain and substrate type. Some strains can simultaneously utilize glucose and fats, carrying out both *de novo* and *ex novo* lipid synthesis [90]. In other strains, exogenous fatty acids may inhibit key enzymes in the *de novo* pathway, but glucose can still be partially utilized and converted into unsaturated fatty acids. Overall, co-substrate cultivation typically results in significantly higher total lipid accumulation than the use of hydrophilic substrates alone. Furthermore, exogenous fatty acids regulate the expression and activity of desaturases and elongases, manifesting as inhibition in some filamentous fungi, while under certain conditions upregulating the expression of specific desaturase genes, thereby altering the degree of unsaturation and chain length of the final lipid products [94].

4. Regulatory Mechanisms of the Biosynthetic Pathways

4.1. Transcriptional Regulation

4.1.1. SNF1-Mediated Transcriptional Regulation of Lipid Metabolism

Sucrose non-fermenting protein 1 (SNF1, known as AMPK in mammals) is a highly conserved energy sensor in eukaryotes that plays a central role in carbon source utilization and lipid metabolism regulation. SNF1 is a heterotrimeric complex composed of α -catalytic, β -regulatory, and γ -regulatory subunits, and its activity is finely regulated by cellular energy status [95]. The regulatory role of SNF1 in lipid accumulation has been preliminarily elucidated in oleaginous filamentous fungi (Figure 2). In *M. circinelloides* cultured on xylose under nitrogen limitation, increasing the carbon-to-nitrogen (C/N) ratio led to decreased xylose consumption and cell growth while lipid accumulation progressively increased, with optimal lipid yield of 4.1 g/L at a C/N ratio of 160:1. The two SNF1 subunits Snf- α 1 and Snf- β participate in lipid metabolism regulation in response to nutritional signals triggered by different exogenous nitrogen sources [96]. The Snf- β subunit significantly enhances lipid accumulation through ACC1 regulation in response to xylose or mixed glucose-xylose sugars, with effects far exceeding those observed with glucose alone [97].

In *M. alpina*, SNF1 complex transcriptional regulation is also influenced by carbon and nitrogen concentrations in the culture medium, suggesting that SNF1 may be a key node linking nutritional signals to lipid accumulation. The SNF1- γ subunit (MaSnf4) is responsible for energy sensing, and its knockout completely abolishes energy conservation mode. In low-glucose medium, the MaSnf4 knockout strain showed fatty acid content similar to that in high-glucose medium, reaching $16 \pm 1.17\%$ of dry cell weight after 96 h of culture [98]. Overexpression of the SNF1 β subunit (MaSip2) significantly promoted glucose uptake, increasing total biomass by 34.1% and ARA yield by 44.8% [99].

4.1.2. The Nitrogen Metabolism Regulator AreA

AreA is a global GATA transcription factor that regulates nitrogen metabolism and plays a central role in nitrogen metabolite repression [100]. Recent studies have revealed that AreA not only regulates nitrogen metabolism but also influences PUFA synthesis through cross-talk with lipid metabolism. In *M. circinelloides*, AreA knockout strains showed significantly increased lipid yields, while AreA overexpression suppressed lipid synthesis (Figure 2) [100]. Further studies demonstrated that AreA regulates nitrogen uptake and metabolism by modulating *MEP2* gene expression (encoding an ammonium transporter), thereby indirectly regulating lipid biosynthesis [101]. AreA knockout reduces sensitivity to nitrogen availability, promotes lipid synthesis, and high C/N ratios

further enhance lipid accumulation [102]. AreA may regulate lipid metabolism through *uga2*, which encodes succinate semialdehyde dehydrogenase in *M. circinelloides*. Deletion of *uga2* may cause carbon-nitrogen metabolic imbalance, shifting carbon flux toward lipid synthesis [103]. These findings reveal important regulatory nodes between nitrogen and lipid metabolism, providing new targets for improving PUFA yields through genetic modification.

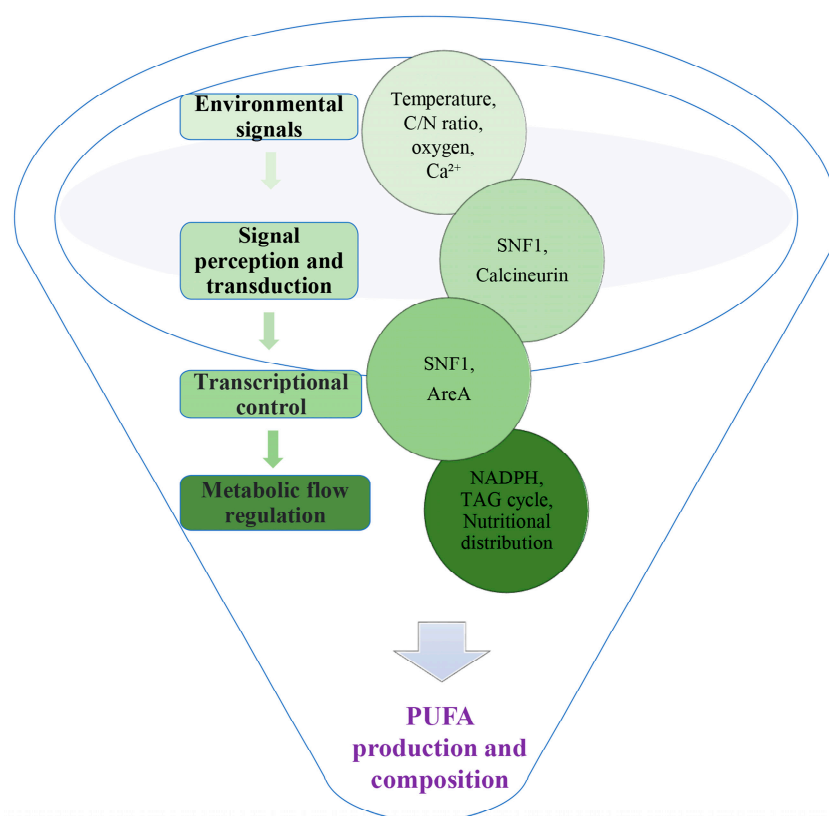


Figure 2. Multi-layered regulatory network governing PUFA synthesis in filamentous fungi. This signaling pathway diagram integrates the regulatory architecture from environmental signals to final PUFA output across four hierarchical levels. Level 1 (environmental signals): temperature, C/N ratio, dissolved oxygen, and Ca²⁺ concentration. Level 2 (signal perception and transduction): the SNF1 energy sensor, Ca²⁺/calcineurin signaling. Level 3 (transcriptional regulation): downstream target genes of SNF1, the nitrogen metabolism global regulator AreA. Level 4 (metabolic flux regulation): nutritional distribution (partitioning of carbon between lipid and carbohydrate synthesis), NADPH supply, as well as triacylglycerol (TAG) cycling. The final output is PUFA yield and composition. The color shades represent different regulatory levels, with lighter colors indicating lower regulatory levels.

4.2. Environmental Regulation

4.2.1. Temperature

Temperature is one of the most important environmental factors affecting PUFA composition in filamentous fungi. As mesophilic fungi, Mortierellaceae species have optimal growth temperatures of 25–28 °C, within which biomass accumulation and most enzymatic reactions involved in PUFA biosynthesis are promoted [104]. Although low temperatures reduce fungal metabolic activity and biomass accumulation, the conversion of ARA to EPA catalyzed by ω -3 desaturase is more favorable at temperatures below 20 °C (Figure 2) [105]. In addition, Zygomycetes filamentous fungus *C. elegans* also exhibits a preference for lower temperatures for GLA accumulation. A study showed that when

strain NRRL Y-1392 was cultured at 12 °C, the GLA content increased to 15.5% *w/w*, compared with only 12.8% *w/w* at 28 °C; however, the yield decreased significantly with prolonged cultivation time [106]. This underscores the importance of monitoring the fermentation process to determine the optimal harvesting point during fermentation process optimization.

4.2.2. Oxygen

PUFA biosynthesis in filamentous fungi is an aerobic process, with biomass and lipid yields significantly affected by aeration, agitation, dissolved oxygen concentration, and cell morphology. Oxygen distribution must be rationally regulated to control mycelial morphology and avoid shear stress (Figure 2) [107]. Dissolved oxygen (DO) concentration is a critical parameter for PUFA biosynthesis, as desaturation reactions require molecular oxygen as an electron acceptor. Under standard fermentation conditions (25–28 °C, 1 atm), the saturation concentration of DO in aqueous media is approximately 8.5–9.0 ppm. The optimal DO range for lipid accumulation in *M. alpina* has been reported to be 10–15% of air saturation (equivalent to approximately 1–1.5 ppm) [108,109]. In industrial production of unsaturated fatty acids by aerobic oleaginous microorganisms, high cell density is essential for ensuring high yields because lipids are intracellular products. However, high biomass concentrations reduce mass transfer efficiency, affecting oxygen transfer and slowing desaturation reaction rates. While increasing agitation, aeration flow rates, and pressure can improve dissolved oxygen (DO) supply efficiency, this approach has limitations due to potential mycelial damage [110]. As a process parameter, the volumetric oxygen transfer coefficient plays a fundamental role in lipid synthesis and scale-up, being related to the combination of agitation intensity, gas surface velocity, and effective liquid viscosity [111,112]. Agitation and aeration directly influence mycelial morphology in stirred tank reactors, causing the morphology to range from dense spherical pellets to viscous mycelium [113]. An increase in agitation intensity and aeration rate can shift pellet morphology from rough to smooth, with a concomitant decrease in mycelial roughness. In several filamentous fungi, agitation intensity has been confirmed to be negatively correlated with pellet size, while in *Ganoderma lucidum*, the aeration rate was found to have no significant effect on pellet size [114,115]. Pellet morphology further affects fermentation performance. First, it limits oxygen transfer; studies have shown that dense mycelial structures impede the diffusion of oxygen and nutrient substrates into the pellet interior. In addition, mycelia of filamentous fungi are highly sensitive to shear stress generated by mechanical agitation, and excessive agitation intensity can lead to hyphal fragmentation and pellet breakage, severely compromising cell viability and product synthesis capacity [116]. To resolve this conflict, researchers have developed various morphology control strategies. For example, in ARA fermentation by *M. alpina*, a multi-stage fermentation strategy combined with continuous feeding culture and precise control of agitation and aeration transformed the pellet morphology into “rice-grain-like” pellets, a morphology conducive to oxygen transfer and biomass accumulation, increasing ARA yield by 7.8% [117]. Other studies have shown that adjusting the osmotic pressure of the medium or adding mineral microparticles to the medium (microparticle-enhanced cultivation, MPEC) can effectively promote changes in mycelial morphology, thereby enhancing the production of secondary metabolites and enzymes; however, this technique is species- and particle-specific [118]. Still other studies have employed airlift bioreactors (ALRs) for cultivating filamentous fungi to maintain optimal gas holdup and volumetric oxygen transfer coefficient [119]. An oxygen carrier such as n-hexadecane can act as a surfactant, reducing water surface tension and increasing the specific interfacial area of gases. Addition of 4% n-hexadecane has been reported to significantly increase dissolved oxygen levels in *M. alpina* and promote biomass accumulation, lipid content, and oil yield [120]. Additionally,

high dissolved oxygen levels can be achieved through genetic engineering. Introduction of the *Vitreoscilla* hemoglobin (VHb) gene into *M. alpina* enhances oxygen utilization efficiency under hypoxic conditions, effectively alleviating oxygen limitation in large-scale fermentation and significantly increasing ARA and total lipid yields [121]. These strategies provide feasible technical approaches for mycelial morphology optimization and oxygen balance in PUFA fermentation by filamentous fungi.

4.2.3. pH

pH is another important parameter affecting cell growth and intracellular metabolite stability in Mortierellaceae [122]. pH changes in the culture medium affect the charge state of cell membranes, thereby influencing enzyme activities, particularly membrane-bound enzymes such as elongases and desaturases (Figure 2). Another pH-sensitive enzyme is ME, which participates in NADPH generation [104]. Typically, pH values in Mortierellaceae cultivation range from 5.5 to 6.5 [117,123,124]. Li et al. (2015) [125] demonstrated that a two-stage pH control strategy effectively improved lipid yield: maintaining pH at 5.5 for the first 48 h, then gradually increasing to 6.5 until the end of cultivation, resulting in a lipid yield of 15.5 g/L for the *M. alpina* strain.

4.2.4. Carbon and Nitrogen Sources and Nutritional Signals

The type and concentration of carbon and nitrogen sources are core nutritional factors affecting lipid accumulation in oleaginous fungi (Figure 2) [126]. In *M. alpina*, a mixture of yeast extract (YE) and sodium nitrate optimized ARA accumulation, with sodium nitrate serving as the primary nitrogen source [127]. Subsequently, similar strategies have been applied to *M. alpina* cultivation. For example, using approximately 10 g/L YE and 3 g/L sodium nitrate achieved ARA yields of 6.9 g/L [128], 8.1 g/L [125], and 13.5 g/L [117] in different studies. The C/N ratio is an important parameter affecting PUFA synthesis. Lipid accumulation in oleaginous microorganisms is typically triggered when nitrogen sources are depleted while carbon sources remain abundant [107,127,129]. This phenomenon is closely related to SNF1 signaling pathway perception of nutritional status [130]. Nitrogen depletion activates AMP deaminase activity to compensate for nitrogen deficiency [131]. This is because when the nitrogen source in the medium is exhausted, amino acid synthesis is blocked, the AMP consumption pathway becomes imbalanced, and AMP deaminase is subsequently activated to catalyze the deamination of AMP to inosine monophosphate (IMP) and free ammonium ions, leading to a rapid decrease in intracellular AMP concentration. However, AMP is an activator of mitochondrial NAD⁺-dependent isocitrate dehydrogenase (NAD⁺-IDH), and a decrease in AMP concentration directly inhibits IDH activity, resulting in citrate accumulation in the mitochondria. The accumulated citrate is then transported to the cytoplasm, where it is cleaved by ATP:citrate lyase (ACL) to generate acetyl-CoA, providing sufficient precursors for fatty acid synthesis. Meanwhile, the elevated AMP/ATP ratio activates the AMP-activated protein kinase (AMPK/SNF1) pathway, which subsequently enhances lipid accumulation by regulating the activity of ACC [131–133]. These effects have been observed during cultivation of the filamentous oleaginous fungi *M. circinelloides* [134] and *M. alpina* [107]. Generally, C/N ratios below 10 favor microbial growth, while higher C/N ratios slow growth and redirect metabolism toward lipid synthesis [135]. In *M. alpina* cultivation, higher lipid yields are achieved at C/N ratios between 15 and 32 [135]. Under nitrogen limitation, *C. elegans* NRRL Y-1392 also exhibits high lipid accumulation capacity, producing oleic acid-rich lipids that account for up to 48% (*w/w*) of dry cell weight [65]. However, recent findings indicate that under certain conditions or in specific strains, nitrogen excess can promote PUFA biosynthesis and increase their proportion. For instance, when *C. elegans* NRRL Y-1392 was cultivated under nitrogen-excess conditions on a medium-resembling stalk

hydrolysates, higher production values for biomass, PUFAs, and GLA were observed (20.3 g/L, 1906 mg/L, and 668 mg/L, respectively), accompanied by high productivity due to short cultivation periods. Furthermore, when the fungus was cultivated on a medium derived from hot water extraction of mushroom stalks supplemented with organic nitrogen sources, the dry biomass reached 14.5 g/L, lipid content reached 1.8 g/L, and GLA accounted for 15% (*w/w*) of cellular lipids [65].

The utilization of organic industrial wastes for microbial cultivation to produce high-value-added metabolites is of great significance for achieving sustainable resource utilization. Glycerol is the principal by-product of biodiesel production. For most filamentous fungi, glucose is a superior substrate to glycerol because of the poor regulation of key enzymes involved in glycerol assimilation (e.g., glycerol kinase and 3-phosphate glycerol dehydrogenase) [90]; however, several Zygomycete strains have shown notable exceptions. Studies have demonstrated that *T. elegans*, *C. elegans*, and *U. ramanniana* can grow efficiently on glycerol as the sole carbon source and accumulate GLA-rich lipids at levels comparable to or even higher than those obtained on glucose. For the *C. elegans* NRRL Y-1392 strain, under nitrogen-excess conditions, GLA production was highest, reaching 224.0 mg/L (productivity equivalent to 2.0 mg/L/h); under low-nitrogen conditions, lipid accumulation was higher (0.59 g/g dry biomass), but the production of PUFAs and GLA did not increase. By optimizing the carbon-to-nitrogen ratio to 18.3 mol/mol, higher production of PUFAs (600 mg/L) and GLA (243 mg/L) was achieved. When the fungus was cultivated at 12 °C, the GLA content increased from 12.8% *w/w* at 28 °C to 15.5% *w/w*; however, due to prolonged cultivation time, the production value decreased significantly. In bioreactor experiments at laboratory scale (working volume $V_w = 1.8$ L; $C/N = 18.3$ mol/mol) and semi-pilot scale ($V_w = 15.0$ L; $C/N = 110.0$ mol/mol), stable and consistent results were obtained, indicating that this bioprocess has potential for industrial application [106]. *T. elegans* cultivated on untreated glycerol produced a lipid content exceeding 40% (*w/w*), of which GLA accounted for 7.3% (*w/w*), with a GLA yield of 664.3 mg/L [136]. Another study reported that *T. elegans* produced 11.6 g/L of oil in glycerol medium, with a lipid content of 71.1% (*w/w*) and a maximum GLA content of 371 mg/L [32]. *U. ramanniana* was flask-cultivated under carbon-limited conditions with approximately 35 g/L glycerol; glycerol was rapidly consumed, and the final total dry cell weight was approximately 16 g/L, with a lipid content of approximately 24% *w/w* and a GLA concentration of 9–13% *w/w*, with a maximum GLA concentration of approximately 430 mg/L [64]. These findings demonstrate that glycerol can serve as an efficient and economically feasible substrate for the production of GLA-rich SCO by specific Zygomycetes, and that strain selection is crucial for efficient glycerol utilization.

Food-processing residues, such as cheese whey, are low-cost and abundant substrates for SCO production by Zygomycetes. However, many fungi metabolize lactose, the principal compound in whey, poorly, and therefore the efficient utilization of cheese whey is also strain-specific. Among the Zygomycetes tested, *U. isabellina* stands out for its exceptional capacity to consume lactose and its high biomass and lipid yields. Vamvakaki et al. (2010) [137] compared three Zygomycetes, *U. isabellina*, *T. elegans*, and *Mucor* sp., for their ability to produce GLA-containing lipids during cultivation on cheese whey. The results showed that *U. isabellina* completely consumed all available lactose and significantly degraded proteins, whereas the other two fungi appeared unable to continue utilizing lactose after protein exhaustion. In a second series of experiments, supplementation of *U. isabellina* cultures with lactose increased the C/N ratio and enhanced fat production. On cheese-whey-based medium, *U. isabellina* ATHUM 2935 achieved the highest DCW of 32.00 g/L, with a lipid content of approximately 25% and a maximum GLA production of 301 mg/L. This DCW value is among the highest levels ever reported for oleaginous Zygomycetes cultivated on cheese whey. For *T. elegans* and *Mucor* sp., the addition of

ammonium sulfate was necessary to promote lactose consumption and biomass production. These findings reveal the strain-specific nature of lactose utilization and indicate that *U. isabellina* is a promising candidate for converting cheese whey into GLA-rich SCO.

Lignocellulosic sugars are a collective term for various sugars obtained from lignocellulosic biomass through hydrolysis and separation, representing an abundant and low-cost substrate for bioconversion. Studies have shown that oleaginous Zygomycetes (e.g., *U. isabellina*, *C. echinulata* and *T. elegans*) can utilize lignocellulosic sugars (mainly xylose) to produce GLA-rich SCO, but carbon flux from xylose can also be diverted toward xylitol production [94,138–140]. For example, in both *U. isabellina* and *T. elegans*, xylitol secretion has been observed when xylose is used as the sole carbon source or in mixtures with glucose. One study investigated the role of glucose and xylose in GLA-rich lipid production by *T. elegans* and found that glucose was the best carbon source in terms of lipid production (15.0 g/L), lipid content (47.1% DCW), and GLA production (1014 mg/L). When glucose and xylose were mixed at a 50:50 ratio, GLA-rich lipid production was optimal, with GLA production reaching 974.9 mg/L, close to the level obtained with pure glucose, and xylitol secretion significantly reduced (only 3.4 g/L), indicating that the presence of glucose effectively suppressed the metabolic diversion of xylose to xylitol. In bioreactors, substrate consumption was faster, and metabolism was more biased toward biomass production [67]. One study found that for *U. isabellina* ATHUM 2935, the lipid yield on xylose (0.10 g/g) was significantly lower than that on glucose (0.22 g/g) [94]. However, another study reported that for *U. isabellina* ATCC 42613, the lipid yields on glucose and xylose were 0.41–0.44 g/g and 0.39–0.43 g/g, respectively, showing little difference [141]. Taken together, these studies indicate that xylose is an alternative carbon source for lipid production, but in most cases it is less efficient. Metabolic flux analysis suggests that enhancing xylitol dehydrogenase activity to promote the conversion of xylitol to xylulose, or overexpressing the phosphoketolase pathway to bypass the xylitol intermediate, may redirect carbon flux toward lipid accumulation [142]. These findings provide a basis for the process design of GLA-rich SCO production using lignocellulosic hydrolysates.

4.3. The Cofactor NADPH

NADPH is the essential reducing power for fatty acid synthesis and desaturation reactions (Figure 2). Multiple enzymatic reactions can generate NADPH [80,143]. Overexpression of key enzymes involved in NADPH supply pathways in *M. alpina*, including G6PD, 6-phosphogluconate dehydrogenase (PGD) [84], isocitrate dehydrogenase (IDH) [144], and ME [88], showed that cytosolic IDH increased both total fatty acid and ARA content by 20%, similar to PGD. Among these, G6PD2 had the most significant effect on fatty acid synthesis, increasing total fatty acid content by 1.7-fold, while ME was more effective in desaturation, increasing ARA content by 1.5-fold compared with the control. Co-overexpression of G6PD2 and ME simultaneously promoted fatty acid synthesis and desaturation. Using continuous fermentation over 96 h, the co-overexpressing strain achieved ARA accumulation of 1.9 ± 0.2 g/(L·d), which was 7.2-fold higher than the *M. alpina* control cultured in flasks [85]. This study confirmed that the pentose phosphate pathway is the primary NADPH donor during fatty acid synthesis in *M. alpina*.

4.4. Calcium Signaling Pathway

Calcium signaling (Ca^{2+}) plays a central role in cellular metabolism and participates in regulating multiple physiological processes, mediated by calcineurin—a calcium/calmodulin-dependent phosphatase composed of catalytic subunit A (Cna) and regulatory subunit B (CAR1). The regulatory role of calcium signaling in lipid accumulation in oleaginous filamentous fungi has attracted increasing attention in recent years (Figure 2). Different calcium concentrations significantly affect biomass formation and lipid yield in

M. circinelloides, indicating that calcium is a key metabolic regulator. Calcium deficiency has been shown to trigger lipid accumulation in Mucorales fungi. The CAR1 plays an important regulatory role in growth and lipid accumulation in *M. circinelloides*. Overexpression and knockout studies showed that CAR1 overexpression increased biomass by 31% and lipid accumulation by 22%, while CAR1 knockout reduced both parameters. Furthermore, CAR1 expression altered the fatty acid profile, revealing a strong negative correlation between palmitic acid and GLA. These results indicate that CAR1 plays an important role in connecting calcium signaling with lipid biosynthesis [145,146].

5. Research Progress on Major PUFA Products

5.1. ARA

5.1.1. Metabolic Engineering Strategies

ARA is the most industrially mature and intensively studied PUFA product from filamentous fungi. Its production chassis is almost entirely dependent on *M. alpina*, particularly the 1S-4 strain. Metabolic engineering for ARA has evolved from “single-gene overexpression” to “systematic modular reconstruction”. Early studies focused on overexpressing $\Delta 6$ - and $\Delta 5$ -desaturases, but with limited efficacy. Zhang et al. (2023) reorganized the elongase/desaturase pathway in *M. alpina* based on its functional roles in ARA synthesis into a “Push” module (responsible for precursor LA and GLA supply, involving $\Delta 12$ - and $\Delta 6$ -desaturases) and a “Pull” module (responsible for DGLA-to-ARA conversion, involving GLELO and $\Delta 5$ -desaturase). Using a 2A peptide-mediated multigene co-expression platform, they achieved synergistic optimization of both modules, balancing intermediate production and consumption. The engineered strain MA5 showed approximately 5-fold improvement in ARA yield in shake-flask fermentation, reaching a final titer of 4.4 g/L, with lipid content increased by 1.2-fold and ARA content increased by 77.6% [147].

ARA synthesis is highly dependent on NADPH. The metabolic pathways involving G6PD, PGD, IDH, and ME all supply NADPH. Overexpression of these enzymes effectively increased intracellular NADPH levels and promoted ARA accumulation. Among these, G6PD2 had the most significant effect on fatty acid synthesis, increasing total fatty acid content by 1.7-fold, while ME was more effective in desaturation, increasing ARA content by 1.5-fold. Co-overexpression of G6PD2 and ME simultaneously promoted fatty acid synthesis and desaturation, achieving an ARA yield of 1.9 ± 0.2 g/(L·d) [85]. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a highly conserved enzyme in glycolysis that catalyzes the conversion of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate while generating NADH. Overexpression of *GAPDH1* or silencing of *GAPDH2* both increased the NADPH/NADP ratio, increasing lipid content by approximately 13% and 23%, respectively [148]. Overexpression of GTP cyclohydrolase I (GTPCH) increased tetrahydrobiopterin (BH4) levels by 7-fold. BH4 then increased NADPH levels through the phenylalanine hydroxylase (PAH) and nitric oxide synthase (NOS) pathways, ultimately enhancing cellular fatty acid synthesis and polyunsaturation [149].

5.1.2. Fermentation Process Optimization

M. alpina ARA fermentation is primarily conducted in submerged fermentation (SmF), with multi-stage environmental control as the core strategy. A two-stage aeration rate strategy (1.0 vvm during growth phase, 1.4 vvm during ARA accumulation phase) achieved an ARA yield of 4.7 g/L, representing a 38.2% improvement. A dynamic temperature control strategy (25 °C during growth phase, linear cooling to 16 °C during ARA accumulation phase) increased ARA content by 37.1%. Combining two-stage aeration rate control with dynamic temperature control achieved the highest ARA yield of 5.8 g/L [150]. A fed-batch fermentation strategy reduced the fermentation period from 7 to 5 days while

increasing ARA productivity from 0.9 to 1.3 g/(L·d). Moreover, four cycles of repeated fed-batch fermentation shortened the total fermentation period from 40 to 26 days, demonstrating that this strategy is a convenient and economical process for commercial ARA-rich oil production [151].

5.2. GLA

GLA is a C18 PUFA of the ω -6 series, synthesized from stearic acid (C18:0) through Δ 9-, Δ 12-, and Δ 6-desaturation reactions via a relatively simple pathway. GLA-producing strains mainly include *M. circinelloides*, *C. echinulata*, *U. ramanniana*, *U. isabellina* and *T. elegans* [12–14,20]. *M. circinelloides* WJ11 has available genomic, proteomic, and metabolomic information and well-established genetic tools, making it a valuable model for studying lipid accumulation mechanisms, but its lipid content (36% dry weight) is still surpassed by several other oleaginous Zygomycetes, particularly *U. isabellina* ATHUM 2935 (83.3% dry weight) and *T. elegans* CCF-1465 (47.1% dry weight) [24].

5.2.1. Metabolic Engineering Strategies

In *M. circinelloides* WJ11, overexpression of Δ 6-desaturase alone significantly increased GLA content (to 21% of total fatty acids); simultaneous overexpression of Δ 6- and Δ 12-desaturases (two genes), and overexpression of Δ 6-, Δ 12-, and Δ 9-desaturases (three genes), increased GLA yields by 1.5-fold and 1.9-fold, respectively, compared with the control strain [152]. Overexpression of the autophagy-related protein ATG8 (*atg8-1* and *atg8-2*) was also confirmed to promote lipid accumulation and GLA production [153]. Overexpression of two cytosolic malic enzyme genes, *cmalA* and *cmalB*, increased total GLA content by 11.42% and 16.85%, respectively [154]. Overexpression of two mitochondrial malic enzyme genes, *malC* and *malD*, increased total GLA content by 16.73% and 46.76%, respectively [155]. Furthermore, transcriptomic and lipidomic analyses of *C. echinulata* revealed that when trehalose was used as the carbon source, Δ 6-desaturase expression was significantly upregulated, and GLA content reached 21% of total lipids, far exceeding the 13% observed under glucose cultivation [13].

5.2.2. Fermentation Process Optimization

In *M. circinelloides*, lipid yield reached 4.1 g/L with xylose as the carbon source under optimized C/N ratio (160:1) [96]. Analysis of fermentation conditions identified optimal abiotic conditions including pH 6.5, temperature 25–26 °C, inoculum 15% (*v/v*), agitation 500 rpm, dissolved oxygen 20–30%, and 120 h of fermentation. Glucose co-feeding provided important reference for developing efficient lipid production fermentation strategies [156].

Aerated and agitated bioreactors are often considered essential for achieving high lipid contents in oleaginous Zygomycetes; however, for several oleaginous Zygomycetes (including *U. isabellina* ATHUM 2935, *T. elegans* CCF-1465, and *C. elegans* NRRL Y-1392), shake-flask cultivation and bioreactor cultivation show little difference in lipid yield and productivity. For example, when *C. elegans* NRRL Y-1392 was used for GLA production with glycerol as the substrate, the results from shake-flask experiments (working volume *V*_w = 1.8 L; C/N = 18.3 mol/mol) were consistent with those from semi-pilot-scale bioreactor experiments (*V*_w = 15.0 L; C/N = 110.0 mol/mol) [106]. When *T. elegans* CCF-1465 was cultivated in a 3-L submerged bioreactor with glucose as the sole carbon source, the lipid and GLA concentrations reached 13.9 g/L and 742 mg/L, respectively, which were comparable to the shake-flask results [67]. When *U. isabellina* ATHUM 2935 was scaled up in a 3-L bioreactor with glucose as the substrate, the biomass obtained was 17.5 g/L, with a lipid content of 12.7 g/L [28]. In shake flasks, this strain achieved a DCW exceeding 35.9 g/L, demonstrating high-cell-density cultivation [157]. These results indicate that for

many oleaginous Zygomycetes, shake-flask cultivation remains an effective and economical tool for screening and process development. However, in industrial production, especially when process intensification is required, bioreactor cultivation remains a key means for precise control of dissolved oxygen, pH, and feeding strategies.

5.3. EPA

5.3.1. Metabolic Engineering Strategies

EPA (20:5 n-3) is a representative ω -3 long-chain polyunsaturated fatty acid with beneficial effects in the treatment of cardiovascular diseases and in anti-inflammatory responses. However, the synthetic capacity of filamentous fungi is extremely limited due to the lack of efficient ω -3 desaturases. Therefore, introducing exogenous ω -3-desaturases through genetic engineering has become a core strategy for achieving efficient EPA production. A study isolated a ω -3 fatty acid desaturase gene, *PaD17*, from *P. aphanidermatum* that exhibits strong Δ -17 desaturase activity and a clear substrate preference for ARA. After transforming *PaD17* into *M. alpina*, the conversion rate of ARA to EPA increased to 49.8%. The recombinant strain produced 617 mg/L EPA at room temperature, and after medium optimization the EPA yield increased to 1.73 g/L, with dry cell weight and total fatty acid content reaching 16.55 g/L and 6.46 g/L, respectively [83]. Okuda et al. (2015) expressed the heterologous Δ 17-desaturase gene *sdd17m* from *Saprolegnia diclina* in *M. alpina* ST1358 (a mutant defective in ω -3-desaturase activity), achieving EPA accumulation at ambient temperature (28 °C). In a 5-L fermenter, EPA accounted for 26.4% of total fatty acids, with an EPA yield of 1.8 g/L. This study was the first to report EPA accumulation in *M. alpina* at ambient temperature, providing a platform technology for industrial EPA production [158]. In addition to the two enzymes mentioned above, ω -3 desaturases from *Phytophthora parasitica* (PPD17) and from oomycetes (*psul* ω 3 and *pmd*17) have also been found to enable efficient conversion in recombinant *M. alpina* strains [159,160]. These studies collectively demonstrate that heterologous expression of ω -3-desaturases with high ARA preference is an effective approach for efficient EPA synthesis in *M. alpina*.

5.3.2. Fermentation Process Optimization

The core challenge in optimizing EPA fermentation processes lies in resolving the conflict between insufficient dissolved oxygen supply and pH mismatch. EPA synthesis involves multiple desaturation reactions and has a high demand for molecular oxygen; however, the dissolved oxygen generated by aeration and agitation in fermenters is insufficient to support cell growth and EPA synthesis. Meanwhile, the optimal pH conditions for cell growth and EPA accumulation are inconsistent. Huang et al. (2020) applied a combined strategy of two-stage pH control and oxygen-enriched air to promote EPA production in the recombinant strain *M. alpina* CCFM698 (capable of EPA production at ambient temperature). After 10 days of fermentation in a 7.5-L fermenter, the biomass reached 41.2 g/L, lipid content was 31.5%, EPA accounted for 26.7% of total lipids, and the final EPA yield reached 3.47 g/L, which is the highest EPA yield reported to date for *M. alpina* [161].

P. irregularis is a promising co-producer of EPA and ARA. Russo et al. (2023) systematically optimized the cultivation conditions for *P. irregularis* using food-industry by-products and wastes as inexpensive nutrient sources, testing various substrates including sugarcane molasses, spent brewer's yeast, cheese whey, and expired orange juice. The results showed that the combination of spent brewer's yeast as the nitrogen source and expired orange juice as the organic carbon source was most effective, with optimized biomass productivity of 14.22 g/L, lipid yield of 2.23 g/L, and EPA concentration of 155 mg/L [37]. This study provides a feasible path for sustainable EPA production using low-cost feedstocks.

5.4. DHA

5.4.1. Metabolic Engineering Strategies

DHA (22:6 n-3) is the longest-chain and most highly unsaturated PUFA in the ω -3 series and plays an important role in infant neurodevelopment and maintenance of retinal function. DHA synthesis requires additional Δ 4-desaturase and C22-elongase, which are naturally absent or exhibit extremely low activity in most filamentous fungi [162]. Therefore, heterologous expression is the core strategy for DHA production. However, reports on heterologous DHA synthesis in filamentous fungi are extremely limited, and multiple challenges remain. For example, DHA has the longest carbon chain and the highest degree of unsaturation, and its synthesis requires multiple elongation and desaturation steps, each of which is constrained by the catalytic efficiency of specific enzymes. Screening for enzyme combinations that can be heterologously expressed, achieve high conversion efficiency, and coordinate metabolic flux remains difficult. In addition, the high degree of unsaturation makes DHA extremely sensitive to oxidative degradation, and measures to protect it from loss during fermentation and downstream processing still need to be explored.

5.4.2. Fermentation Process Optimization

Currently, research on DHA synthesis by filamentous fungi (e.g., *Mortierella*) has mainly focused on strain screening, metabolic pathway elucidation, and optimization of basic culture conditions (e.g., temperature, carbon and nitrogen sources). For example, a study isolated *M. alpina* CFR-GV15 from soil in the Western Ghats of India and found that it could accumulate DHA at $4.09 \pm 0.1\%$ of total fatty acids in a lipid production basal medium, without involving fermentation process optimization specifically for DHA [163].

Current experience in DHA fermentation process optimization comes mainly from *Schizochytrium*, the dominant platform for commercial DHA production, including high-density fed-batch fermentation, staged dissolved oxygen control, and scale-up strategies based on the volumetric oxygen transfer coefficient (kLa) [112,164,165]. These experiences are instructive for developing DHA fermentation processes in filamentous fungi; however, they cannot be directly applied because of fundamental differences between filamentous fungi and *Schizochytrium* in cell morphology and hydrodynamic properties. Future work should, on the basis of breakthroughs in strain engineering, simultaneously develop DHA fermentation processes tailored to the morphological characteristics of filamentous fungi.

5.5. DGLA

5.5.1. Metabolic Engineering Strategies

DGLA (20:3 n-6) is the direct precursor of ARA biosynthesis and also possesses anti-inflammatory and anti-proliferative bioactivities, making it a useful raw material for the synthesis of eicosanoid drugs. However, DGLA accumulation in natural microorganisms is generally low because it is rapidly converted to ARA by Δ 5-desaturase. Tamano et al. (2019) integrated the enzyme genes involved in DGLA synthesis from *M. alpina* as a gene cluster into the chromosome of an *A. oryzae* strain with a disrupted acyl-CoA synthetase gene, and knocked out the Δ 5-desaturase gene that converts DGLA to ARA. The constructed engineered strain produced 145 mg/L of free DGLA after 5 days of cultivation. Upon addition of 1% Triton X-100 surfactant to the culture, more than 80% of the free DGLA was released extracellularly without affecting cell growth. This was the first report of microbial production of free DGLA and its extracellular release [42]. Subsequently, on this basis, overexpression of a predicted transketolase gene and disruption of the α -1,3-glucan synthase gene further increased DGLA production to 533 mg/L, significantly enhancing the capacity of the *A. oryzae* cell factory for DGLA production [17].

5.5.2. Fermentation Process Optimization

Surfactant-assisted product release is a key strategy for optimizing DGLA fermentation in *A. oryzae*. The addition of 1% Triton X-100 achieved an extracellular release rate of more than 80% without inhibiting cell growth, greatly simplifying the downstream processing and avoiding the use of lipases to release free DGLA from triacylglycerols. In addition, fed-batch fermentation and carbon-to-nitrogen (C/N) ratio optimization based on this strain are under further investigation [42].

6. Challenges and Future Directions

6.1. Current Major Technical Bottlenecks

Industrialization of filamentous fungal PUFA production is constrained by three core bottlenecks: Genetic manipulation difficulties include the multinucleate/heterokaryotic nature and low homologous recombination efficiency of filamentous fungi make it difficult to achieve homozygous mutations through precise gene editing, restricting systematic strain improvement [166–168]; low synthesis efficiency of very long-chain PUFAs (especially DHA, C22:6 ω -3)—some filamentous fungi naturally lack ω -3 pathway enzyme systems [169] or can only be weakly induced to produce ω -3 PUFAs under specific environmental stress [170], and DHA titers in filamentous fungi remain substantially lower than those achieved with commercial *Schizochytrium* platforms, a thraustochytrid stramenopile that employs an oxygen-independent PUFA synthase pathway and is currently the dominant microbial source of commercial DHA [171]; prominent fermentation engineering challenges—the non-Newtonian fluid properties and shear sensitivity of mycelia make it difficult to balance oxygen mass transfer and cell viability in large-scale fermentation [119], and the long fermentation cycle significantly increases production costs and operational risks [151].

6.2. Emerging Technologies and Future Research Directions

Optimization of CRISPR gene editing technology is the core breakthrough for overcoming genetic manipulation bottlenecks, requiring the development of delivery systems better suited for filamentous fungi and multi-round homozygosis strategies [172]. Multi-omics integration and systems metabolic engineering can systematically identify rate-limiting nodes and reconstruct complex metabolic pathways by integrating genomic, transcriptomic, proteomic, and metabolomic data, combined with genome-scale metabolic models and the 2A peptide multigene co-expression platform [147,173,174]. Development of sustainable feedstocks and solid-state fermentation focuses on reducing carbon source costs using agricultural and industrial wastes as inexpensive substrates, while exploiting the unique advantages of solid-state fermentation in enhancing PUFA content [175,176]. Discovery and domestication of novel strains aims to transform under-explored PUFA-producing filamentous fungi from nature into industrial platform strains through high-throughput screening and systematic genetic domestication [60,177].

Beyond their direct use as nutritional supplements in food, health products, feed, and other applications, GLA-rich SCOs produced by oleaginous Zygomycetes can also be converted into fatty acid potassium or lithium salts (FAPs/FALs). These fatty acid alkali salts are water-soluble and bio-assimilable, overcoming the limitation of poor aqueous solubility of PUFAs and enabling their application in pharmaceutical formulations. Studies have shown that FALS from *C. echinulata* (containing 11–18% GLA) exhibit cytotoxic and genotoxic effects against HL-60 leukemia cells, inducing DNA fragmentation at high concentrations [178]. FALS from glycerol-cultured *C. elegans* (containing ~9.5% GLA) are cytotoxic to PC-3 and DU-145 prostate cancer cells, as well as TPC-1 and K1 thyroid cancer cells, with IC₅₀ values of ~58–61 μ g/mL [179]. FAPS from *T. elegans* (containing 6.5% GLA)

exhibit marked lethal effects against MCF-7 breast cancer cells with an IC_{50} of 0.3 $\mu\text{g/mL}$ [180]. FALS from *T. elegans* and *M. alpina* suppress the proliferation and migration of PC-3 and DU-145 prostate cancer cells [181]. The anticancer mechanisms of FAPs/FALs involve multiple pathways, including induction of oxidative stress, lipid peroxidation, and modulation of cell signaling cascades. The conversion of FAPs/FALs utilizes crude SCOs (rather than purified GLA), offering a promising and cost-effective route for producing high-value-added pharmaceuticals from microbial oils.

6.3. Industrialization Prospects and Sustainability Considerations

Successful industrialization of filamentous fungal PUFAs depends on the coordinated achievement of three economic indicators: product value, productivity, and feedstock cost [104]. Compared with traditional fish oil and algal oil, the filamentous fungal platform offers competitive advantages including year-round production, low contamination risk, substrate flexibility, and relatively simplified downstream processing, but still faces severe cost pressures in large-scale competition [72,182,183]. Future competitiveness enhancement will rely on continuous strain performance iteration, fermentation process engineering innovation, and product differentiation strategies such as targeted production of specific PUFA components [19,150,151]. Furthermore, regulatory approval and safety assessment of novel strains and products—including toxicological, allergenicity, and genetic stability evaluations—are necessary safeguards for industrialization [11,184]. With continuously growing global demand for sustainable PUFA sources and supportive biomanufacturing policies, filamentous fungal PUFAs are expected to achieve broader applications in food, pharmaceutical, and feed sectors, and to provide a transferable paradigm for microbial synthesis of other high-value lipid compounds.

7. Conclusions

PUFAs play critical physiological roles in cardiovascular health, neurodevelopment, and immune regulation, with continuously growing market demand. However, traditional fish oil and plant-based PUFA supply chains face severe challenges including resource depletion, environmental pollution, and sustainability crises. Against this backdrop, using filamentous fungi for single cell oil (SCO) production through microbial fermentation has emerged as a sustainable and economically viable alternative. This review provides a comprehensive overview of research progress on PUFA synthesis by filamentous fungi, covering strain resources, biosynthetic pathways, regulatory mechanisms, development status of major PUFA products, and industrialization prospects, outlining a comprehensive landscape from fundamental biological understanding to engineering applications.

In terms of key research advances, filamentous fungi have formed a complete spectrum from native high-yielding strains to genetically tractable chassis cells: ARA-rich oil derived from *M. alpina* has been authorized by the FDA and the EU as a food ingredient; *M. circinelloides* is a model strain for GLA production; and *A. oryzae* provides an ideal chassis for heterologous expression. Regarding biosynthetic pathways, filamentous fungi begin with acetyl-CoA, synthesize saturated fatty acids through the FAS system, and then generate ARA, EPA, and other PUFAs through sequential catalysis by Δ^9 -, Δ^{12} -, Δ^6 -, and Δ^5 -desaturases and elongases. At the regulatory level, the SNF1 energy sensor perceives carbon and nitrogen nutritional status, AreA links nitrogen metabolism with lipid synthesis, temperature and other environmental factors modulate fatty acid unsaturation by influencing desaturase gene expression, and carbon allocation and NADPH supply collectively affect PUFA yields. In metabolic engineering, genetic modification has evolved from single-gene manipulation to systematic engineering, with the 2A peptide-based multigene co-expression platform increasing ARA production approximately 5-fold, and

cofactor engineering and competitive pathway blockade enabling targeted production of specific PUFAs, while *A. oryzae* cell factory modification ultimately achieved a DGLA titer of 533 mg. In fermentation technology, fed-batch fermentation and multi-stage strategies with segmented temperature, oxygen, and agitation control significantly improved target product yields.

Nevertheless, industrialization of filamentous fungal PUFA production still faces three core challenges: genetic manipulation difficulties, low synthesis efficiency of DHA and other very long-chain PUFAs, and prominent fermentation engineering challenges. Looking forward, continued optimization of CRISPR gene editing will overcome genetic modification bottlenecks; multi-omics integration and systems biology will provide comprehensive metabolic regulatory insights; systems metabolic engineering and synthetic biology tools will enable a paradigm shift from “single-gene manipulation” to “systematic reconstruction”; and sustainable feedstock utilization and novel strain discovery and domestication will fundamentally improve economic viability. With synergistic advancement of these directions, the potential of filamentous fungi as “lipid cell factories” for PUFAs will be further unlocked.

Author Contributions: Conceptualization was performed by Y.Y.; literature data were collected by Y.Y., Z.Y., M.Z., W.L., J.W. and Y.Z.; writing—original draft preparation by Y.Y., Z.Y., M.Z., W.L. and J.W.; writing—review and editing by Y.Y. and M.Z.; funding acquisition, Y.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This project was supported by grants from the National Key Laboratory Major Special Project, China (25ZXZSS00350).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: During the preparation of this manuscript/study, the authors used DeepSeek Web Version (DeepSeek-V4.1-Flash) for literature analysis of Chapter 6 Challenges and Future Directions of this review. The authors have reviewed and edited the output and take full responsibility for the content of this publication.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Das, U.N. Essential fatty acids—A review. *Curr. Pharm. Biotechnol.* **2006**, *7*, 467–482. <https://doi.org/10.2174/138920106779116856>.
2. Bellou, S.; Triantaphyllidou, I.E.; Aggeli, D.; Elazzazy, A.M.; Baeshen, M.N.; Aggelis, G. Microbial oils as food additives: Recent approaches for improving microbial oil production and its polyunsaturated fatty acid content. *Curr. Opin. Biotechnol.* **2016**, *37*, 24–35. <https://doi.org/10.1016/j.copbio.2015.09.005>.
3. Khan, I.; Hussain, M.; Jiang, B.; Zheng, L.; Pan, Y.; Hu, J.; Khan, A.; Ashraf, A.; Zou, X. Omega-3 long-chain polyunsaturated fatty acids: Metabolism and health implications. *Prog. Lipid Res.* **2023**, *92*, 101255. <https://doi.org/10.1016/j.plipres.2023.101255>.
4. Poli, A.; Agostoni, C.; Visioli, F. Dietary fatty acids and inflammation: Focus on the n-6 series. *Int. J. Mol. Sci.* **2023**, *24*, 4567. <https://doi.org/10.3390/ijms24054567>.
5. Dalaka, E.; Vasilakis, G.; Bilbilai, M.; Karayannis, D.; Sanida, M.; Politis, I.; Diamantopoulou, P.; Papanikolaou, S.; Theodorou, G. Cunninghamella elegans cultivation on agro-industrial residues and assessment of immunomodulatory properties of bioproducts in THP-1 macrophages. *Clean Technol.* **2026**, *8*, 61.
6. Derbyshire, E.J.; Birch, C.S.; Bonwick, G.A.; English, A.; Metcalfe, P.; Li, W. Optimal omegas—Barriers and novel methods to narrow omega-3 gaps. A narrative review. *Front. Nutr.* **2024**, *11*, 1325099. <https://doi.org/10.3389/fnut.2024.1325099>.

7. Shahidi, F.; Ambigaipalan, P. Omega-3 polyunsaturated fatty acids and their health benefits. *Annu. Rev. Food Sci. Technol.* **2018**, *9*, 345–381. <https://doi.org/10.1146/annurev-food-111317-095850>.
8. Shah, A.M.; Yang, W.; Mohamed, H.; Zhang, Y.; Song, Y. Microbes: A hidden treasure of polyunsaturated fatty acids. *Front. Nutr.* **2022**, *9*, 827837. <https://doi.org/10.3389/fnut.2022.827837>.
9. Xie, D.; Jackson, E.N.; Zhu, Q. Sustainable source of omega-3 eicosapentaenoic acid from metabolically engineered *Yarrowia lipolytica*: From fundamental research to commercial production. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 1599–1610. <https://doi.org/10.1007/s00253-014-6318-y>.
10. Yan, C.X.; Zhang, Y.; Yang, W.Q.; Ma, W.; Sun, X.M.; Huang, H. Universal and unique strategies for the production of polyunsaturated fatty acids in industrial oleaginous microorganisms. *Biotechnol. Adv.* **2024**, *70*, 108298. <https://doi.org/10.1016/j.biotechadv.2023.108298>.
11. Chang, L.; Lu, H.; Chen, H.; Tang, X.; Zhao, J.; Zhang, H.; Chen, Y.Q.; Chen, W. Lipid metabolism research in oleaginous fungus *Mortierella alpina*: Current progress and future prospects. *Biotechnol. Adv.* **2022**, *54*, 107794. <https://doi.org/10.1016/j.biotechadv.2021.107794>.
12. Fazili, A.B.A.; Shah, A.M.; Zan, X.; Naz, T.; Nosheen, S.; Nazir, Y.; Ullah, S.; Zhang, H.; Song, Y. *Mucor circinelloides*: A model organism for oleaginous fungi and its potential applications in bioactive lipid production. *Microb. Cell Fact.* **2022**, *21*, 29. <https://doi.org/10.1186/s12934-022-01758-9>.
13. Li, S.; Yue, Q.; Zhou, S.; Yan, J.; Zhang, X.; Ma, F. Trehalose contributes to gamma-linolenic acid accumulation in *Cunninghamella echinulata* based on de novo transcriptomic and lipidomic analyses. *Front. Microbiol.* **2018**, *9*, 1296. <https://doi.org/10.3389/fmicb.2018.01296>.
14. Fakas, S.; Certik, M.; Papanikolaou, S.; Aggelis, G.; Komaitis, M.; Galiotou-Panayotou, M. Gamma-linolenic acid production by *Cunninghamella echinulata* growing on complex organic nitrogen sources. *Bioresour. Technol.* **2008**, *99*, 5986–5990. <https://doi.org/10.1016/j.biortech.2007.10.016>.
15. Papanikolaou, S.; Diamantopoulou, P.; Chatzifragkou, A.; Philippoussis, A.; Aggelis, G. Suitability of low-cost sugars as substrates for lipid production by the fungus *Thamnidium elegans*. *Energy Fuels* **2010**, *24*, 4078–4086. <https://doi.org/10.1021/ef1004804>.
16. Schoch, C.L.; Ciufo, S.; Domrachev, M.; Hottot, C.L.; Kannan, S.; Khovanskaya, R.; Leipe, D.; Mcveigh, R.; O'Neill, K.; Robbertse, B.; et al. NCBI Taxonomy: A comprehensive update on curation, resources and tools. *Database* **2020**, *2020*, baaa062. <https://doi.org/10.1093/database/baaa062>.
17. Tamano, K.; Nakai, S.; Takayama, H.; Imai, Y. Overexpression of a predicted transketolase gene and disruption of an α -1,3-glucan synthase gene in *Aspergillus oryzae* DGLA3 strain enhances the yield of free dihomog- γ -linolenic acid. *Biosci. Biotechnol. Biochem.* **2023**, *87*, 448–457. <https://doi.org/10.1093/bbb/zbad003>.
18. Kikukawa, H.; Sakuradani, E.; Ando, A.; Shimizu, S.; Ogawa, J. Arachidonic acid production by the oleaginous fungus *Mortierella alpina* 1S-4: A review. *J. Adv. Res.* **2018**, *11*, 15–22. <https://doi.org/10.1016/j.jare.2018.02.003>.
19. Sakuradani, E. Advances in the production of various polyunsaturated fatty acids through oleaginous fungus *Mortierella alpina* breeding. *Biosci. Biotechnol. Biochem.* **2010**, *74*, 908–917. <https://doi.org/10.1271/bbb.100001>.
20. Amano, N.; Shinmen, Y.; Akimoto, K.; Kawashima, H.; Amachi, T. Chemotaxonomic significance of fatty acid composition in the genus *Mortierella* (*Zygomycetes*, *Mortierellaceae*). *Mycotaxon* **1992**, *44*, 257–265.
21. Ratledge, C.; Wynn, J.P. The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. *Adv. Appl. Microbiol.* **2002**, *51*, 1–51. [https://doi.org/10.1016/s0065-2164\(02\)51000-5](https://doi.org/10.1016/s0065-2164(02)51000-5).
22. Ratledge, C. Single cell oils—have they a biotechnological future? *Trends Biotechnol.* **1993**, *11*, 278–284. [https://doi.org/10.1016/0167-7799\(93\)90015-2](https://doi.org/10.1016/0167-7799(93)90015-2).
23. Wagner, L.; Stielow, J.B.; De Hoog, G.S.; Bensch, K.; Schwartze, V.U.; Voigt, K.; Alastruey-Izquierdo, A.; Kurzai, O.; Walther, G. A new species concept for the clinically relevant *Mucor circinelloides* complex. *Persoonia* **2020**, *44*, 67–97. <https://doi.org/10.3767/persoonia.2020.44.03>.
24. Tang, X.; Chen, H.; Chen, Y.Q.; Chen, W.; Garre, V.; Song, Y.; Ratledge, C. Comparison of biochemical activities between high and low lipid-producing strains of *Mucor circinelloides*: An explanation for the high oleaginicacy of strain WJ11. *PLoS ONE* **2015**, *10*, e0128396. <https://doi.org/10.1371/journal.pone.0128396>.
25. Tang, X.; Zhao, L.; Chen, H.; Chen, Y.Q.; Chen, W.; Song, Y.; Ratledge, C. Complete genome sequence of a high lipid-producing strain of *Mucor circinelloides* WJ11 and comparative genome analysis with a low lipid-producing strain CBS 277.49. *PLoS ONE* **2015**, *10*, e0137543. <https://doi.org/10.1371/journal.pone.0137543>.

26. Tang, X.; Chen, H.; Gu, Z.; Zhang, H.; Chen, Y.Q.; Song, Y.; Chen, W. Comparative proteome analysis between high lipid-producing strain *Mucor circinelloides* WJ11 and low lipid-producing strain CBS 277.49. *J. Agric. Food Chem.* **2017**, *65*, 5074–5082. <https://doi.org/10.1021/acs.jafc.7b00935>.
27. Tang, X.; Zhang, H.; Chen, H.; Chen, Y.Q.; Chen, W.; Song, Y. Effects of 20 standard amino acids on the growth, total fatty acids production, and γ -linolenic acid yield in *Mucor circinelloides*. *Curr. Microbiol.* **2014**, *69*, 899–908. <https://doi.org/10.1007/s00284-014-0671-z>.
28. Chatzifragkou, A.; Fakas, S.; Galiotou-Panayotou, M.; Komaitis, M.; Aggelis, G.; Papanikolaou, S. Commercial sugars as substrates for lipid accumulation in *Cunninghamella echinulata* and *Mortierella isabellina* fungi. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 1048–1057. <https://doi.org/10.1002/ejlt.201000027>.
29. Dyal, S.D.; Narine, S.S. Implications for the use of *Mortierella* fungi in the industrial production of essential fatty acids. *Food Res. Int.* **2005**, *38*, 445–467. <https://doi.org/10.1016/j.foodres.2004.11.002>.
30. Papanikolaou, S.; Rontou, M.; Belka, A.; Athenaki, M.; Gardeli, C.; Mallouchos, A.; Kalantzi, O.; Koutinas, A.A.; Kookos, I.K.; Zeng, A.P.; et al. Conversion of biodiesel-derived glycerol into biotechnological products of industrial significance by yeast and fungal strains. *Eng. Life Sci.* **2017**, *17*, 262–281. <https://doi.org/10.1002/elsc.201500191>.
31. Papanikolaou, S.; Aggelis, G. Sources of microbial oils with emphasis to *Mortierella (Umbelopsis) isabellina* fungus. *World J. Microbiol. Biotechnol.* **2019**, *35*, 63. <https://doi.org/10.1007/s11274-019-2631-z>.
32. Chatzifragkou, A.; Makri, A.; Belka, A.; Bellou, S.; Mavrou, M.; Mastoridou, M.; Mystrioti, P.; Onjaro, G.; Aggelis, G.; Papanikolaou, S. Biotechnological conversions of biodiesel derived waste glycerol by yeast and fungal species. *Energy* **2011**, *36*, 1097–1108. <https://doi.org/10.1016/j.energy.2010.11.040>.
33. Lio, J.; Wang, T. *Pythium irregulare* fermentation to produce arachidonic acid (ARA) and eicosapentaenoic acid (EPA) using soybean processing co-products as substrates. *Appl. Biochem. Biotechnol.* **2013**, *169*, 595–611. <https://doi.org/10.1007/s12010-012-0032-y>.
34. Cheng, M.; Walker, T.; Hulbert, G.J.; Raman, D.R. Fungal production of eicosapentaenoic and arachidonic acids from industrial waste streams and crude soybean oil. *Bioresour. Technol.* **1999**, *67*, 101–110.
35. Hong, H.; Datla, N.; Mackenzie, S.L.; Qiu, X. Isolation and characterization of a $\Delta 5$ FA desaturase from *Pythium irregulare* by heterologous expression in *Saccharomyces cerevisiae* and oilseed crops. *Lipids* **2002**, *37*, 863–868. <https://doi.org/10.1007/s11745-002-0972-5>.
36. Hong, H.; Datla, N.; Reed, D.W.; Covello, P.S.; Mackenzie, S.L.; Qiu, X. High-level production of gamma-linolenic acid in *Brassica juncea* using a $\Delta 6$ desaturase from *Pythium irregulare*. *Plant Physiol.* **2002**, *129*, 354–362. <https://doi.org/10.1104/pp.001495>.
37. Russo, G.; Langellotti, A.; Martín-García, B.; Verardo, V.; Romano, R.; Sacchi, R.; Masi, P. New biotechnological production of EPA by *Pythium irregulare* using alternative sustainable media obtained from food industry by-products and waste. *Sustainability* **2023**, *15*, 1147. <https://doi.org/10.3390/su15021147>.
38. Kendrick, A.; Ratledge, C. Lipids of selected molds grown for production of n-3 and n-6 polyunsaturated fatty acids. *Lipids* **1992**, *27*, 15–20. <https://doi.org/10.1007/bf02537052>.
39. Machida, M.; Asai, K.; Sano, M.; Tanaka, T.; Kumagai, T.; Terai, G.; Kusumoto, K.; Arima, T.; Akita, O.; Kashiwagi, Y.; et al. Genome sequencing and analysis of *Aspergillus oryzae*. *Nature* **2005**, *438*, 1157–1161. <https://doi.org/10.1038/nature04300>.
40. Tamano, K. Advancements in lipid production research using the koji-mold *Aspergillus oryzae* and future outlook. *Front. Fungal Biol.* **2024**, *5*, 1526568. <https://doi.org/10.3389/ffunb.2024.1526568>.
41. Frisvad, J.C.; Møller, L.L.H.; Larsen, T.O.; Kumar, R.; Arnau, J. Safety of the fungal workhorses of industrial biotechnology: Update on the mycotoxin and secondary metabolite potential of *Aspergillus niger*, *Aspergillus oryzae*, and *Trichoderma reesei*. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 9481–9515. <https://doi.org/10.1007/s00253-018-9354-1>.
42. Tamano, K.; Cox, R.S., 3rd; Tsuge, K.; Miura, A.; Itoh, A.; Ishii, J.; Tamura, T.; Kondo, A.; Machida, M. Heterologous production of free dihomogamma-linolenic acid by *Aspergillus oryzae* and its extracellular release via surfactant supplementation. *J. Biosci. Bioeng.* **2019**, *127*, 451–457. <https://doi.org/10.1016/j.jbiosc.2018.09.013>.
43. Nagamine, S.; Liu, C.; Nishishita, J.; Kozaki, T.; Sogahata, K.; Sato, Y.; Minami, A.; Ozaki, T.; Schmidt-Dannert, C.; Maruyama, J.I.; et al. Ascomycete *Aspergillus oryzae* Is an efficient expression host for production of basidiomycete terpenes by using genomic DNA sequences. *Appl. Environ. Microbiol.* **2019**, *85*, e00409-19. <https://doi.org/10.1128/aem.00409-19>.
44. Qi, J.; Han, H.; Sui, D.; Tan, S.; Liu, C.; Wang, P.; Xie, C.; Xia, X.; Gao, J.M.; Liu, C. Efficient production of a cyclic dipeptide (cyclo-TA) using heterologous expression system of filamentous fungus *Aspergillus oryzae*. *Microb. Cell Fact.* **2022**, *21*, 146. <https://doi.org/10.1186/s12934-022-01872-8>.

45. Han, H.; Yu, C.; Qi, J.; Wang, P.; Zhao, P.; Gong, W.; Xie, C.; Xia, X.; Liu, C. High-efficient production of mushroom polyketide compounds in a platform host *Aspergillus oryzae*. *Microb. Cell Fact.* **2023**, *22*, 60. <https://doi.org/10.1186/s12934-023-02071-9>.
46. Tamano, K.; Yasunaka, Y.; Kamishima, M.; Itoh, A.; Miura, A.; Kan, E.; Koyama, Y.; Tamura, T. Enhancement of the productivity of free dihomogamma-linolenic acid via co-overexpression of elongase and two desaturase genes in *Aspergillus oryzae*. *J. Biosci. Bioeng.* **2020**, *130*, 480–488. <https://doi.org/10.1016/j.jbiosc.2020.07.004>.
47. Zhang, S.; Sato, H.; Ichinose, S.; Tanaka, M.; Miyazawa, K.; Yoshimi, A.; Abe, K.; Shintani, T.; Gomi, K. Cell wall α -1,3-glucan prevents α -amylase adsorption onto fungal cell in submerged culture of *Aspergillus oryzae*. *J. Biosci. Bioeng.* **2017**, *124*, 47–53. <https://doi.org/10.1016/j.jbiosc.2017.02.013>.
48. Chutrakul, C.; Jeennor, S.; Panchanawaporn, S.; Cheawchanlertfa, P.; Suttiwattanakul, S.; Veerana, M.; Laoteng, K. Metabolic engineering of long chain-polyunsaturated fatty acid biosynthetic pathway in oleaginous fungus for dihomogamma linolenic acid production. *J. Biotechnol.* **2016**, *218*, 85–93. <https://doi.org/10.1016/j.jbiotec.2015.12.003>.
49. Anantayanon, J.; Jeennor, S.; Panchanawaporn, S.; Chutrakul, C.; Laoteng, K. Significance of two intracellular triacylglycerol lipases of *Aspergillus oryzae* in lipid mobilization: A perspective in industrial implication for microbial lipid production. *Gene* **2021**, *793*, 145745. <https://doi.org/10.1016/j.gene.2021.145745>.
50. Chiang, Y.M.; Lin, T.S.; Wang, C.C.C. Total heterologous biosynthesis of fungal natural products in *Aspergillus nidulans*. *J. Nat. Prod.* **2022**, *85*, 2484–2518. <https://doi.org/10.1021/acs.jnatprod.2c00487>.
51. Wu, C.C.; Stierle, A.A.; Stierle, D.B.; Chen, H.; Swyers, M.; Decker, T.; Borkowski, E.; Korajczyk, P.; Ye, R.; Mondava, N. Activation of cryptic biosynthetic gene clusters by fungal artificial chromosomes to produce novel secondary metabolites. *AIMS Microbiol.* **2023**, *9*, 757–779. <https://doi.org/10.3934/microbiol.2023039>.
52. Bok, J.W.; Ye, R.; Clevenger, K.D.; Mead, D.; Wagner, M.; Krowiec, A.; Albright, J.C.; Goering, A.W.; Thomas, P.M.; Kelleher, N.L.; et al. Fungal artificial chromosomes for mining of the fungal secondary metabolome. *BMC Genom.* **2015**, *16*, 343. <https://doi.org/10.1186/s12864-015-1561-x>.
53. Kumari, P.; Sharma, J.; Khare, P. Recent advancements and strategies for omega-3 fatty acid production in yeast. *J. Basic. Microbiol.* **2025**, *65*, e2400491. <https://doi.org/10.1002/jobm.202400491>.
54. Gemperlein, K.; Dietrich, D.; Kohlstedt, M.; Zipf, G.; Bernauer, H.S.; Wittmann, C.; Wenzel, S.C.; Müller, R. Polyunsaturated fatty acid production by *Yarrowia lipolytica* employing designed myxobacterial PUFA synthases. *Nat. Commun.* **2019**, *10*, 4055. <https://doi.org/10.1038/s41467-019-12025-8>.
55. Cordova, L.T.; Alper, H.S. Production of α -linolenic acid in *Yarrowia lipolytica* using low-temperature fermentation. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 8809–8816. <https://doi.org/10.1007/s00253-018-9349-y>.
56. Chuang, L.T.; Chen, D.C.; Nicaud, J.M.; Madzak, C.; Chen, Y.H.; Huang, Y.S. Co-expression of heterologous desaturase genes in *Yarrowia lipolytica*. *New Biotechnol.* **2010**, *27*, 277–282. <https://doi.org/10.1016/j.nbt.2010.02.006>.
57. Sun, M.L.; Madzak, C.; Liu, H.H.; Song, P.; Ren, L.J.; Huang, H.; Ji, X.J. Engineering *Yarrowia lipolytica* for efficient γ -linolenic acid production. *Biochem. Eng. J.* **2017**, *117*, 172–180. <https://doi.org/10.1016/j.bej.2016.10.014>.
58. Liu, H.H.; Wang, C.; Lu, X.Y.; Huang, H.; Tian, Y.; Ji, X.J. Improved production of arachidonic acid by combined pathway engineering and synthetic enzyme fusion in *Yarrowia lipolytica*. *J. Agric. Food Chem.* **2019**, *67*, 9851–9857. <https://doi.org/10.1021/acs.jafc.9b03727>.
59. Wang, J.; Yu, X.; Wang, K.; Lin, L.; Liu, H.H.; Ledesma-Amaro, R.; Ji, X.J. Reprogramming the fatty acid metabolism of *Yarrowia lipolytica* to produce the customized omega-6 polyunsaturated fatty acids. *Bioresour. Technol.* **2023**, *383*, 129231. <https://doi.org/10.1016/j.biortech.2023.129231>.
60. Kosa, G.; Zimmermann, B.; Kohler, A.; Ekeberg, D.; Afseth, N.K.; Mounier, J.; Shapaval, V. High-throughput screening of Micromycota fungi for production of low- and high-value lipids. *Biotechnol. Biofuels* **2018**, *11*, 66. <https://doi.org/10.1186/s13068-018-1070-7>.
61. Hashem, A.H.; Abu-Elreesh, G.; El-Sheikh, H.H.; Suleiman, W.B. Isolation, identification, and statistical optimization of a psychrotolerant *Mucor racemosus* for sustainable lipid production. *Biomass Convers. Biorefin.* **2023**, *13*, 3415–3426. <https://doi.org/10.1007/s13399-022-02390-8>.
62. De Rossi, P.; Ambrico, A.; Del Fiore, A.; Trupo, M.; Blasi, L.; Beccaccioli, M.; Faino, L.; Ceci, A.; Maggi, O.; Persiani, A.M.; et al. Antarctic fungi: A bio-source alternative to produce polyunsaturated fatty acids (PUFAs). *Microbiol. Spectr.* **2026**, *14*, e0137225. <https://doi.org/10.1128/spectrum.01372-25>.
63. Ramirez-Castrillon, M.; Benavides-León, T.A.; Arcos-Velasco, L.V.; Pantoja-Pulido, K.D.; Lopez-Parra, L.L.; Bolaños-Rojas, A.C.; Osorio-Cadavid, E. Tropical lakes as a novel source of oleaginous yeasts with lipid profiles for biodiesel, oleochemical, and nutraceutical applications. *World J. Microbiol. Biotechnol.* **2025**, *41*, 105. <https://doi.org/10.1007/s11274-025-04309-7>.

64. Melanouri, E.M.; Papanikolaou, S.; Diamantopoulou, P. *Mortierella ramanniana* lipid fermentation wastewater as an innovative maceration liquid medium for sustainable solid-state cultivation of higher fungi. *Waste Biomass Valor.* **2024**, *12*, 15. <https://doi.org/10.1007/s12649-024-02575-4>.
65. Vasilakis, G.; Gamraoui, A.; Karayannis, D.; Giannakis, N.; Chatti, A.; Politis, I.; Diamantopoulou, P.; Papanikolaou, S. Mycelial mass, microbial lipids and γ -linolenic acid (GLA) by *Cunninghamella elegans* cultivated on agro-industrial residues. *Resour. Chem. Mater.* **2025**, *4*, 100083. <https://doi.org/10.1016/j.recmm.2024.10.001>.
66. Sukrutha, S.K.; Adamechova, Z.; Rachana, K.; Savitha, J.; Certik, M. Optimization of physiological growth conditions for maximal gamma-linolenic acid production by *Cunninghamella blakesleeana*-JSK2. *J. Am. Oil Chem. Soc.* **2014**, *91*, 1507–1513. <https://doi.org/10.1007/s11746-014-2507-1>.
67. Zikou, E.; Chatzifragkou, A.; Koutinas, A.A.; Papanikolaou, S. Evaluating glucose and xylose as cosubstrates for lipid accumulation and gamma-linolenic acid biosynthesis of *Thamnidium elegans*. *J. Appl. Microbiol.* **2013**, *114*, 1020–1032. <https://doi.org/10.1111/jam.12116>.
68. Metz, J.G.; Roessler, P.; Facciotti, D.; Levering, C.; Dittrich, F.; Lassner, M.; Valentine, R.; Lardizabal, K.; Domergue, F.; Yamada, A.; et al. Production of polyunsaturated fatty acids by polyketide synthases in both prokaryotes and eukaryotes. *Science* **2001**, *293*, 290–293. <https://doi.org/10.1126/science.1059593>.
69. Santín, O.; Moncalián, G. Loading of malonyl-CoA onto tandem acyl carrier protein domains of polyunsaturated fatty acid synthases. *J. Biol. Chem.* **2018**, *293*, 12491–12501. <https://doi.org/10.1074/jbc.RA118.002443>.
70. Qiu, X.; Xie, X.; Meesapyodsuk, D. Molecular mechanisms for biosynthesis and assembly of nutritionally important very long chain polyunsaturated fatty acids in microorganisms. *Prog. Lipid Res.* **2020**, *79*, 101047. <https://doi.org/10.1016/j.plipres.2020.101047>.
71. Gong, Y.; Wan, X.; Jiang, M.; Hu, C.; Hu, H.; Huang, F. Metabolic engineering of microorganisms to produce omega-3 very long-chain polyunsaturated fatty acids. *Prog. Lipid Res.* **2014**, *56*, 19–35. <https://doi.org/10.1016/j.plipres.2014.07.001>.
72. Kothri, M.; Mavrommati, M.; Elazzazy, A.M.; Baeshen, M.N.; Moussa, T.A.A.; Aggelis, G. Microbial sources of polyunsaturated fatty acids (PUFAs) and the prospect of organic residues and wastes as growth media for PUFA-producing microorganisms. *FEMS Microbiol. Lett.* **2020**, *367*, fnaa028. <https://doi.org/10.1093/femsle/fnaa028>.
73. Cardenas, J.; Da Silva, N.A. Engineering cofactor and transport mechanisms in *Saccharomyces cerevisiae* for enhanced acetyl-CoA and polyketide biosynthesis. *Metab. Eng.* **2016**, *36*, 80–89. <https://doi.org/10.1016/j.ymben.2016.02.009>.
74. Dulermo, T.; Lazar, Z.; Dulermo, R.; Rakicka, M.; Haddouche, R.; Nicaud, J.M. Analysis of ATP-citrate lyase and malic enzyme mutants of *Yarrowia lipolytica* points out the importance of mannitol metabolism in fatty acid synthesis. *Biochim. Biophys. Acta* **2015**, *1851*, 1107–1117. <https://doi.org/10.1016/j.bbalip.2015.04.007>.
75. Qin, N.; Li, L.; Ji, X.; Li, X.; Zhang, Y.; Larsson, C.; Chen, Y.; Nielsen, J.; Liu, Z. Rewiring central carbon metabolism ensures increased provision of Acetyl-CoA and NADPH required for 3-OH-propionic acid production. *ACS Synth. Biol.* **2020**, *9*, 3236–3244. <https://doi.org/10.1021/acssynbio.0c00264>.
76. Yao, L.; Shen, H.; Wang, N.; Tatlay, J.; Li, L.; Tan, T.W.; Lee, Y.K. Elevated acetyl-CoA by amino acid recycling fuels microalgal neutral lipid accumulation in exponential growth phase for biofuel production. *Plant Biotechnol. J.* **2017**, *15*, 497–509. <https://doi.org/10.1111/pbi.12648>.
77. Morabito, C.; Bournaud, C.; Maës, C.; Schuler, M.; Aiese Cigliano, R.; Dellero, Y.; Maréchal, E.; Amato, A.; Rébeillé, F. The lipid metabolism in thraustochytrids. *Prog. Lipid Res.* **2019**, *76*, 101007. <https://doi.org/10.1016/j.plipres.2019.101007>.
78. Zhu, Z.; Zhang, S.; Liu, H.; Shen, H.; Lin, X.; Yang, F.; Zhou, Y.J.; Jin, G.; Ye, M.; Zou, H.; et al. A multi-omic map of the lipid-producing yeast *Rhodospiridium toruloides*. *Nat. Commun.* **2012**, *3*, 1112. <https://doi.org/10.1038/ncomms2112>.
79. Zhu, Z.; Zhou, Y.J.; Krivoruchko, A.; Grninger, M.; Zhao, Z.K.; Nielsen, J. Expanding the product portfolio of fungal type I fatty acid synthases. *Nat. Chem. Biol.* **2017**, *13*, 360–362. <https://doi.org/10.1038/nchembio.2301>.
80. Ratledge, C. Regulation of lipid accumulation in oleaginous micro-organisms. *Biochem. Soc. Trans.* **2002**, *30*, 1047–1050. <https://doi.org/10.1042/bst0301047>.
81. Zhao, H.; Nie, Y.; Jiang, Y.; Wang, S.; Zhang, T.Y.; Liu, X.Y. Comparative genomics of mortierellaceae provides insights into lipid metabolism: Two novel types of fatty acid synthase. *J. Fungi* **2022**, *8*, 891. <https://doi.org/10.3390/jof8090891>.
82. Certik, M.; Shimizu, S. Kinetic analysis of oil biosynthesis by an arachidonic acid-producing fungus, *Mortierella alpina* 1S-4. *Appl. Microbiol. Biotechnol.* **2000**, *54*, 224–230. <https://doi.org/10.1007/s002530000361>.
83. Ge, C.; Chen, H.; Mei, T.; Tang, X.; Chang, L.; Gu, Z.; Zhang, H.; Chen, W.; Chen, Y.Q. Application of a ω -3 desaturase with an arachidonic acid preference to eicosapentaenoic acid production in *Mortierella alpina*. *Front. Bioeng. Biotechnol.* **2017**, *5*, 89. <https://doi.org/10.3389/fbioe.2017.00089>.

84. Chen, H.; Hao, G.; Wang, L.; Wang, H.; Gu, Z.; Liu, L.; Zhang, H.; Chen, W.; Chen, Y.Q. Identification of a critical determinant that enables efficient fatty acid synthesis in oleaginous fungi. *Sci. Rep.* **2015**, *5*, 11247. <https://doi.org/10.1038/srep11247>.
85. Hao, G.; Chen, H.; Gu, Z.; Zhang, H.; Chen, W.; Chen, Y.Q. Metabolic engineering of *Mortierella alpina* for enhanced arachidonic acid production through the NADPH-supplying strategy. *Appl. Environ. Microbiol.* **2016**, *82*, 3280–3288. <https://doi.org/10.1128/aem.00572-16>.
86. Wasylenko, T.M.; Ahn, W.S.; Stephanopoulos, G. The oxidative pentose phosphate pathway is the primary source of NADPH for lipid overproduction from glucose in *Yarrowia lipolytica*. *Metab. Eng.* **2015**, *30*, 27–39. <https://doi.org/10.1016/j.ymben.2015.02.007>.
87. Koh, H.J.; Lee, S.M.; Son, B.G.; Lee, S.H.; Ryoo, Z.Y.; Chang, K.T.; Park, J.W.; Park, D.C.; Song, B.J.; Veech, R.L.; et al. Cytosolic NADP⁺-dependent isocitrate dehydrogenase plays a key role in lipid metabolism. *J. Biol. Chem.* **2004**, *279*, 39968–39974. <https://doi.org/10.1074/jbc.M402260200>.
88. Rodríguez-Frómata, R.A.; Gutiérrez, A.; Torres-Martínez, S.; Garre, V. Malic enzyme activity is not the only bottleneck for lipid accumulation in the oleaginous fungus *Mucor circinelloides*. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 3063–3072. <https://doi.org/10.1007/s00253-012-4432-2>.
89. Xue, J.; Balamurugan, S.; Li, D.W.; Liu, Y.H.; Zeng, H.; Wang, L.; Yang, W.D.; Liu, J.S.; Li, H.Y. Glucose-6-phosphate dehydrogenase as a target for highly efficient fatty acid biosynthesis in microalgae by enhancing NADPH supply. *Metab. Eng.* **2017**, *41*, 212–221. <https://doi.org/10.1016/j.ymben.2017.04.008>.
90. Papanikolaou, S.; Aggelis, G. Lipids of oleaginous yeasts. Part I: Biochemistry of single cell oil production. *Eur. J. Lipid Sci. Technol.* **2011**, *113*, 1031–1051. <https://doi.org/10.1002/ejlt.201100014>.
91. Patel, A.; Matsakas, L. A comparative study on de novo and ex novo lipid fermentation by oleaginous yeast using glucose and sonicated waste cooking oil. *Ultrason. Sonochem.* **2019**, *52*, 364–374. <https://doi.org/10.1016/j.ultsonch.2018.12.010>.
92. Čertík, M.; Baltészov, L.; Šajbidor, J. Lipid formation and γ -linolenic acid production by Mucorales fungi grown on sunflower oil. *Lett. Appl. Microbiol.* **2010**, *25*, 101–105. <https://doi.org/10.1046/j.1472-765X.1997.00173.x>.
93. Kendrick, A.; Ratledge, C. Cessation of polyunsaturated fatty acid formation in four selected filamentous fungi when grown on plant oils. *J. Am. Oil Chem. Soc.* **1996**, *73*, 431–435. <https://doi.org/10.1007/BF02523914>.
94. Athenaki, M.; Gardeli, C.; Diamantopoulou, P.; Tchakouteu, S.S.; Sarris, D.; Philippoussis, A.; Papanikolaou, S. Lipids from yeasts and fungi: Physiology, production and analytical considerations. *J. Appl. Microbiol.* **2018**, *124*, 336–367. <https://doi.org/10.1111/jam.13633>.
95. Nosheen, S.; Naz, T.; Yang, J.; Hussain, S.A.; Fazili, A.B.A.; Nazir, Y.; Li, S.; Mohamed, H.; Yang, W.; Mustafa, K.; et al. Role of Snf- β in lipid accumulation in the high lipid-producing fungus *Mucor circinelloides* WJ11. *Microb. Cell Fact.* **2021**, *20*, 52. <https://doi.org/10.1186/s12934-021-01545-y>.
96. Zhang, Y.; Yang, Y.; Zhang, S.; Liu, Q.; Dang, W.; Song, Y. Lipid accumulation and SNF1 transcriptional analysis of *Mucor circinelloides* on xylose under nitrogen limitation. *Antonie Van Leeuwenhoek* **2023**, *116*, 383–391. <https://doi.org/10.1007/s10482-023-01810-7>.
97. Zhang, Y.; Yang, Y.; Liu, Q.; Li, S.; Song, Y. Lipid accumulation by Snf- β engineered *Mucor circinelloides* strains on glucose and xylose. *Appl. Biochem. Biotechnol.* **2023**, *195*, 7697–7707. <https://doi.org/10.1007/s12010-023-04531-9>.
98. Chang, L.; Tang, X.; Zhang, H.; Chen, Y.Q.; Chen, H.; Chen, W. Improved lipogenesis in *Mortierella alpina* by abolishing the Snf4-mediated energy-saving mode under low glucose. *J. Agric. Food Chem.* **2020**, *68*, 10787–10798. <https://doi.org/10.1021/acs.jafc.0c04572>.
99. Chang, L.; Tang, X.; Zhang, H.; Chen, Y.Q.; Chen, H.; Chen, W. SNF1 β -modulated glucose uptake and the balance between polyunsaturated fatty acids and carbohydrates in *Mortierella alpina*. *J. Agric. Food Chem.* **2021**, *69*, 13849–13858. <https://doi.org/10.1021/acs.jafc.1c05971>.
100. Hu, H.; Li, P.; Li, S.; Wang, X.; Mohamed, H.; López-García, S.; Liu, Q.; Garre, V.; Song, Y. The role of areA in lipid accumulation in high lipid-producing fungus *Mucor circinelloides* WJ11. *Biochim. Biophys. Acta. Mol. Cell Biol. Lipids* **2024**, *1869*, 159450. <https://doi.org/10.1016/j.bbalip.2023.159450>.
101. Wang, X.; Mohamed, H.; Hina, K.; Liu, Q.; Fan, Z.; Song, Y. Role of AreA in regulating lipid metabolism through modulation of MEP2 in the oleaginous fungus *Mucor circinelloides*. *Fungal Biol.* **2025**, *68*, 106476.
102. Wang, X.; Li, S.; Pang, S.; Liu, Q.; Song, Y. Regulation of AreA on lipid biosynthesis under different nitrogen sources and C/N ratios in the model oleaginous fungus *Mucor circinelloides*. *Biochim. Biophys. Acta. Mol. Cell Biol. Lipids* **2024**, *1869*, 159537. <https://doi.org/10.1016/j.bbalip.2024.159537>.

103. Wang, X.; Mohamed, H.; Liu, Q.; Fan, Z.; Song, Y. The nitrogen regulator AreA modulates lipid metabolism through *uga2* in *Mucor circinelloides*. *Fungal Biol.* **2025**, *129*, 101616. <https://doi.org/10.1016/j.funbio.2025.101616>.
104. Chang, L.; Chen, H.; Tang, X.; Zhao, J.; Zhang, H.; Chen, Y.Q.; Chen, W. Advances in improving the biotechnological application of oleaginous fungus *Mortierella alpina*. *Appl. Microbiol. Biotechnol.* **2021**, *105*, 6275–6289. <https://doi.org/10.1007/s00253-021-11480-y>.
105. Sakuradani, E.; Abe, T.; Iguchi, K.; Shimizu, S. A novel fungal omega3-desaturase with wide substrate specificity from arachidonic acid-producing *Mortierella alpina* 1S-4. *Appl. Microbiol. Biotechnol.* **2005**, *66*, 648–654. <https://doi.org/10.1007/s00253-004-1760-x>.
106. Vasilakis, G.; Roidouli, C.; Karayannis, D.; Giannakis, N.; Rondags, E.; Chevalot, I.; Papanikolaou, S. Study of different parameters affecting production and productivity of polyunsaturated fatty acids (PUFAs) and γ -linolenic acid (GLA) by *Cunninghamella elegans* through glycerol conversion in shake flasks and bioreactors. *Microorganisms* **2024**, *12*, 2097.
107. Lu, H.; Chen, H.; Tang, X.; Yang, Q.; Zhang, H.; Chen, Y.Q.; Chen, W. Metabolomics analysis reveals the role of oxygen control in the nitrogen limitation induced lipid accumulation in *Mortierella alpina*. *J. Biotechnol.* **2021**, *325*, 325–333. <https://doi.org/10.1016/j.jbiotec.2020.10.004>.
108. Higashiyama, K.; Fujikawa, S.; Park, E.Y.; Shimizu, S. Production of arachidonic acid by *Mortierella* fungi. *Biotechnol. Bioprocess. Eng.* **2002**, *7*, 252–262.
109. Palaiogeorgou, A.M.; Papanikolaou, S.; De Castro, A.M.; Freire, D.M.G.; Kookos, I.K.; Koutinas, A.A. A newly isolated *Enterobacter* sp. strain produces 2,3-butanediol during its cultivation on low-cost carbohydrate-based substrates. *FEMS Microbiol. Lett.* **2019**, *366*, fny280. <https://doi.org/10.1093/femsle/fny280>.
110. Zhang, X.; Jiang, L.; Zhu, L.Y.; Shen, Q.K.; Ji, X.J.; Huang, H.; Zhang, H.M. Effects of aeration on metabolic profiles of *Mortierella alpina* during the production of arachidonic acid. *J. Ind. Microbiol. Biotechnol.* **2017**, *44*, 1225–1235. <https://doi.org/10.1007/s10295-017-1950-z>.
111. García-Ochoa, F.; Castro, E.G.; Santos, V.E. Oxygen transfer and uptake rates during xanthan gum production. *Enzym. Microb. Technol.* **2000**, *27*, 680–690. [https://doi.org/10.1016/s0141-0229\(00\)00272-6](https://doi.org/10.1016/s0141-0229(00)00272-6).
112. Qu, L.; Ren, L.-J.; Huang, H. Scale-up of docosahexaenoic acid production in fed-batch fermentation by *Schizochytrium* sp. based on volumetric oxygen-transfer coefficient. *Biochem. Eng. J.* **2013**, *77*, 82–87. <https://doi.org/10.1016/j.bej.2013.05.011>.
113. Krull, R.; Wucherpfennig, T.; Esfandabadi, M.E.; Walisko, R.; Melzer, G.; Hempel, D.C.; Kampen, I.; Kwade, A.; Wittmann, C. Characterization and control of fungal morphology for improved production performance in biotechnology. *J. Biotechnol.* **2013**, *163*, 112–123. <https://doi.org/10.1016/j.jbiotec.2012.06.024>.
114. Nair, R.B.; Lennartsson, P.R.; Taherzadeh, M.J. Mycelial pellet formation by edible ascomycete filamentous fungi, *Neurospora intermedia*. *AMB. Express* **2016**, *6*, 31. <https://doi.org/10.1186/s13568-016-0203-2>.
115. Nielsen, J.; Johansen, C.L.; Jacobsen, M.; Krabben, P.; Villadsen, J. Pellet formation and fragmentation in submerged cultures of *Penicillium chrysogenum* and its relation to penicillin production. *Biotechnol. Progress.* **1995**, *11*, 93–98. <https://doi.org/10.1021/bp00031a013>.
116. Wucherpfennig, T.; Kiep, K.A.; Driouch, H.; Wittmann, C.; Krull, R. Morphology and rheology in filamentous cultivations. *Adv. Appl. Microbiol.* **2010**, *72*, 89–136. [https://doi.org/10.1016/s0065-2164\(10\)72004-9](https://doi.org/10.1016/s0065-2164(10)72004-9).
117. Wu, W.J.; Zhang, A.H.; Peng, C.; Ren, L.J.; Song, P.; Yu, Y.D.; Huang, H.; Ji, X.J. An efficient multi-stage fermentation strategy for the production of microbial oil rich in arachidonic acid in *Mortierella alpina*. *Bioresour. Bioprocess.* **2017**, *4*, 8. <https://doi.org/10.1186/s40643-017-0138-8>.
118. Antecka, A.; Bizukojc, M.; Ledakowicz, S. Modern morphological engineering techniques for improving productivity of filamentous fungi in submerged cultures. *World J. Microbiol. Biotechnol.* **2016**, *32*, 193. <https://doi.org/10.1007/s11274-016-2148-7>.
119. Cerrone, F.; O'Connor, K.E. Cultivation of filamentous fungi in airlift bioreactors: Advantages and disadvantages. *Appl. Microbiol. Biotechnol.* **2025**, *109*, 41. <https://doi.org/10.1007/s00253-025-13422-4>.
120. Peng, C.; Huang, H.; Ji, X.; Liu, X.; Ren, L.; Yu, W.; You, J.; Lu, J. Effects of n-hexadecane concentration and a two-stage oxygen supply control strategy on arachidonic acid production by *Mortierella Alpina* ME-1. *Chem. Eng. Technol.* **2010**, *33*, 692–697. <https://doi.org/10.1002/ceat.200900413>.
121. Zhang, H.; Feng, Y.; Cui, Q.; Song, X. Expression of Vitreoscilla hemoglobin enhances production of arachidonic acid and lipids in *Mortierella alpina*. *BMC Biotechnol.* **2017**, *17*, 68. <https://doi.org/10.1186/s12896-017-0388-8>.
122. Mironov, A.A.; Nemashkalov, V.A.; Stepanova, N.N.; Kamzolova, S.V.; Rymowicz, W.; Morgunov, I.G. The effect of pH and temperature on arachidonic acid production by glycerol-grown *Mortierella alpina* NRRL-A-10995. *Fermentation* **2018**, *4*, 17. <https://doi.org/10.3390/FERMENTATION4010017>.

123. Jin, M.J.; Huang, H.; Xiao, A.H.; Zhang, K.; Liu, X.; Li, S.; Peng, C. A novel two-step fermentation process for improved arachidonic acid production by *Mortierella alpina*. *Biotechnol. Lett.* **2008**, *30*, 1087–1091. <https://doi.org/10.1007/s10529-008-9661-1>.
124. Malaiwong, N.; Yongmanitchai, W.; Chonudomkul, D. Optimization of arachidonic acid production from *Mortierella alpina* PRAO7-10 by response surface methodology. *Agric. Nat. Resour.* **2016**, *50*, 162–172.
125. Li, X.; Lin, Y.; Chang, M.; Jin, Q.; Wang, X. Efficient production of arachidonic acid by *Mortierella alpina* through integrating fed-batch culture with a two-stage pH control strategy. *Bioresour. Technol.* **2015**, *181*, 275–282. <https://doi.org/10.1016/j.biortech.2015.01.009>.
126. Lu, H.; Chen, H.; Tang, X.; Yang, Q.; Zhang, H.; Chen, Y.Q.; Chen, W. Ultra performance liquid chromatography-q exactive orbitrap/mass spectrometry-based lipidomics reveals the influence of nitrogen sources on lipid biosynthesis of *Mortierella alpina*. *J. Agric. Food Chem.* **2019**, *67*, 10984–10993. <https://doi.org/10.1021/acs.jafc.9b04455>.
127. Lu, J.; Peng, C.; Ji, X.-J.; You, J.; Cong, L.; Ouyang, P.; Huang, H. Fermentation characteristics of *Mortierella alpina* in response to different nitrogen sources. *Appl. Biochem. Biotechnol.* **2011**, *164*, 979–990. <https://doi.org/10.1007/s12010-011-9189-z>.
128. Yu, Y.; Zhang, L.; Li, T.; Wu, N.; Jiang, L.; Ji, X.; Huang, H. How nitrogen sources influence *Mortierella alpina* aging: From the lipid droplet proteome to the whole-cell proteome and metabolome. *J. Proteom.* **2018**, *179*, 140–149. <https://doi.org/10.1016/j.jprot.2018.03.014>.
129. Li, L.; Jin, N. Effect of nitrogen concentration on the growth and fatty acid content of *Mortierella alpina*. *Int. J. Agric. Nat. Resour.* **2020**, *24*, 838–848.
130. Zhang, Y.; Yang, Y.; Zhang, H.; Liu, Q.; Song, Y. Effect of different carbons on lipid production and SNF1 transcription in *Mucor Circinelloides*. *Indian. J. Microbiol.* **2023**, *63*, 146–151. <https://doi.org/10.1007/s12088-023-01070-z>.
131. Chang, L.; Tang, X.; Lu, H.; Zhang, H.; Chen, Y.Q.; Chen, H.; Chen, W. Role of adenosine monophosphate deaminase during fatty acid accumulation in oleaginous fungus *Mortierella alpina*. *J. Agric. Food Chem.* **2019**, *67*, 9551–9559. <https://doi.org/10.1021/acs.jafc.9b03603>.
132. Li, S.; Yang, J.; Mohamed, H.; Wang, X.; Shi, W.; Xue, F.; López-García, S.; Liu, Q.; Song, Y. AMP deaminase: A crucial regulator in nitrogen stress and lipid metabolism in *Mucor circinelloides*. *Biochim. Biophys. Acta. Mol. Cell Biol. Lipids* **2024**, *1869*, 159434. <https://doi.org/10.1016/j.bbalip.2023.159434>.
133. Li, S.; Tahiri, G.; Yang, J.; Mohamed, H.; Liu, Q.; Shi, W.; López-García, S.; Garre, V.; Song, Y. Role of AMP deaminase in *Mucor circinelloides*: Implications for nitrogen utilization and lipid biosynthesis. *J. Agric. Food Chem.* **2023**, *71*, 15680–15691. <https://doi.org/10.1021/acs.jafc.3c04574>.
134. Zhao, L.; Zhang, H.; Wang, L.; Chen, H.; Chen, Y.Q.; Chen, W.; Song, Y. (13)C-metabolic flux analysis of lipid accumulation in the oleaginous fungus *Mucor circinelloides*. *Bioresour. Technol.* **2015**, *197*, 23–29. <https://doi.org/10.1016/j.biortech.2015.08.035>.
135. Koike, Y.; Cai, H.J.; Higashiyama, K.; Fujikawa, S.; Park, E.Y. Effect of consumed carbon to nitrogen ratio of mycelial morphology and arachidonic acid production in cultures of *Mortierella alpina*. *J. Biosci. Bioeng.* **2001**, *91*, 382–389. <https://doi.org/10.1263/jbb.91.382>.
136. Fakas, S.; Bellou, S.; Makri, A.; Aggelis, G. Single cell oil and gamma-linolenic acid production by *Thamnidium elegans* grown on raw glycerol. In *Microbial Conversions of Raw Glycerol*; Aggelis, G., Ed.; Nova Science Publishers: New York, NY, USA, 2009.
137. Vamvakaki, A.N.; Kandarakis, I.; Kaminarides, S.; Komaitis, M.; Papanikolaou, S. Cheese whey as a renewable substrate for microbial lipid and biomass production by Zygomycetes. *Eng. Life Sci.* **2010**, *10*, 348–360. <https://doi.org/10.1002/elsc.201000063>.
138. Jia, Y.; Yang, W.; Zhang, L.; Hu, X.; Zhang, H.; Zhang, B. Xylitol biomanufacturing: Production technologies, industrial applications and future opportunities. *Fermentation* **2026**, *12*, 366.
139. Gardeli, C.; Athenaki, M.; Xenopoulos, E.; Mallouchos, A.; Koutinas, A.A.; Aggelis, G.; Papanikolaou, S. Lipid production and characterization by *Mortierella (Umbelopsis) isabellina* cultivated on lignocellulosic sugars. *J. Appl. Microbiol.* **2017**, *123*, 1461–1477. <https://doi.org/10.1111/jam.13587>.
140. Fakas, S.; Papanikolaou, S.; Batsos, A.; Galiotou-Panayotou, M.; Mallouchos, A.; Aggelis, G. Evaluating renewable carbon sources as substrates for single cell oil production by *Cunninghamella echinulata* and *Mortierella isabellina*. *Biomass Bioenerg.* **2009**, *33*, 573–580. <https://doi.org/10.1016/j.biombioe.2008.09.006>.
141. Ruan, Z.; Zanolli, M.; Wang, X.; Ducey, C.; Liu, Y. Evaluation of lipid accumulation from lignocellulosic sugars by *Mortierella isabellina* for biodiesel production. *Bioresour. Technol.* **2012**, *110*, 198–205. <https://doi.org/10.1016/j.biortech.2012.01.053>.
142. Pandey, N.; Ahmad, D.; Hasan, M.; Choudhary, D.K.; Kumar, A.; Tripathi, M.K.; Siddiqui, S.A.; Shah, M.A. Current trends in the production of xylitol and paving the way for metabolic engineering in microbes. *Biotechnol. Biofuels Bioprod.* **2025**, *18*, 106. <https://doi.org/10.1186/s13068-025-02702-w>.

143. Zhang, Y.; Adams, I.P.; Ratledge, C. 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* **2007**, *153*, 2013–2025. <https://doi.org/10.1099/mic.0.2006/002683-0>.
144. Tang, X.; Sun, X.; Wang, X.; Zhang, H.; Chen, Y.Q.; Zhao, J.; Chen, H.; Chen, W. Characterization of NAD⁽⁺⁾/NADP⁽⁺⁾-specific isocitrate dehydrogenases from oleaginous fungus *Mortierella alpina* involved in lipid accumulation. *Front. Nutr.* **2021**, *8*, 746342. <https://doi.org/10.3389/fnut.2021.746342>.
145. Baalsrud, H.T.; Byrtusova, D.; To, T.H.; Larsen, I.E.; Bøe, V.A.; Grønvold, L.; Fu, J.; Árnýasi, M.; Shapaval, V.; Sandve, S.R. Calcium starvation leads to strain-specific gene regulation of lipid and carotenoid production in *Mucor circinelloides*. *G3* **2025**, *15*, jkaf207. <https://doi.org/10.1093/g3journal/jkaf207>.
146. Ramadan, A.S.; Mohamed, H.; Xu, H.; Khalid, H.; Eltoukhy, A.; Naz, T.; Du, C.; Iqbal, B.; Song, Y. Functional role of calcineurin regulatory subunit B (CAR1) in lipid accumulation in oleaginous fungus *Mucor circinelloides*. *Bioresour. Technol.* **2026**, *453*, 134684. <https://doi.org/10.1016/j.biortech.2026.134684>.
147. Zhang, H.; Wan, W.; Cui, Q.; Song, X. Modular metabolic engineering of *Mortierella alpina* by the 2A peptide platform to improve arachidonic acid production. *J. Agric. Food Chem.* **2023**, *71*, 12519–12527. <https://doi.org/10.1021/acs.jafc.3c03016>.
148. Wang, S.; Chen, H.; Tang, X.; Zhang, H.; Hao, G.; Chen, W.; Chen, Y.Q. The role of glyceraldehyde-3-phosphate dehydrogenases in NADPH supply in the oleaginous filamentous fungus *Mortierella alpina*. *Front. Microbiol.* **2020**, *11*, 818. <https://doi.org/10.3389/fmicb.2020.00818>.
149. Wang, H.; Zhang, C.; Chen, H.; Gu, Z.; Zhao, J.; Zhang, H.; Chen, Y.Q.; Chen, W. Tetrahydrobiopterin plays a functionally significant role in lipogenesis in the oleaginous fungus *Mortierella alpina*. *Front. Microbiol.* **2020**, *11*, 250. <https://doi.org/10.3389/fmicb.2020.00250>.
150. Gao, M.J.; Wang, C.; Zheng, Z.Y.; Zhu, L.; Zhan, X.B.; Lin, C.C. Improving arachidonic acid fermentation by *Mortierella alpina* through multistage temperature and aeration rate control in bioreactor. *Prep. Biochem. Biotechnol.* **2016**, *46*, 360–367. <https://doi.org/10.1080/10826068.2015.1031397>.
151. Ji, X.J.; Zhang, A.H.; Nie, Z.K.; Wu, W.J.; Ren, L.J.; Huang, H. Efficient arachidonic acid-rich oil production by *Mortierella alpina* through a repeated fed-batch fermentation strategy. *Bioresour. Technol.* **2014**, *170*, 356–360. <https://doi.org/10.1016/j.biortech.2014.07.098>.
152. Wang, X.; Yang, J.; Mohamed, H.; Shah, A.M.; Li, S.; Pang, S.; Wu, C.; Xue, F.; Shi, W.; Sadaqat, B.; et al. Simultaneous overexpression of $\Delta 6$ -, $\Delta 12$ - and $\Delta 9$ -desaturases enhanced the production of γ -linolenic acid in *Mucor circinelloides* WJ11. *Front. Microbiol.* **2022**, *13*, 1078157. <https://doi.org/10.3389/fmicb.2022.1078157>.
153. Li, H.; Yuan, H.; Iqbal, B.; Wang, T.; Wang, Z.; Zhang, H. The role of atg8 in promoting lipid accumulation in the oleaginous fungus *Mucor circinelloides* during nitrogen limitation. *J. Fungi* **2026**, *12*, 410. <https://doi.org/10.3390/jof12060410>.
154. Fazili, A.B.A.; Shah, A.M.; Naz, T.; Nosheen, S.; Yang, W.; Garre, V.; Majeed, Y.; Al-Sadoon, M.K.; Song, Y. Role of cytosolic malic enzyme in oleaginicacy of high-lipid-producing fungal strain *Mucor circinelloides* WJ11. *J. Fungi* **2022**, *8*, 265. <https://doi.org/10.3390/jof8030265>.
155. Fazili, A.B.A.; Shah, A.M.; Albeshr, M.F.; Naz, T.; Dar, M.A.; Yang, W.; Garre, V.; Fazili, K.M.; Bhat, E.A.; Song, Y. Overexpression of the mitochondrial malic enzyme genes (*malC* and *malD*) improved the lipid accumulation in *Mucor circinelloides* WJ11. *Front. Microbiol.* **2022**, *13*, 919364. <https://doi.org/10.3389/fmicb.2022.919364>.
156. Mohamed, H.; Naz, T.; Liu, Q.; Li, S.; Wang, X.; Song, Y. Fed-batch fermentation of *Mucor circinelloides* reveals significant improvement in biomass and lipid accumulation through performance evaluation, chemical analysis, and expression profiling. *Bioresour. Technol.* **2024**, *398*, 130540. <https://doi.org/10.1016/j.biortech.2024.130540>.
157. Papanikolaou, S.; Komaitis, M.; Aggelis, G. Single cell oil (SCO) production by *Mortierella isabellina* grown on high-sugar content media. *Bioresour. Technol.* **2004**, *95*, 287–291. <https://doi.org/10.1016/j.biortech.2004.02.016>.
158. Okuda, T.; Ando, A.; Negoro, H.; Muratsubaki, T.; Kikukawa, H.; Sakamoto, T.; Sakuradani, E.; Shimizu, S.; Ogawa, J. Eicosapentaenoic acid (EPA) production by an oleaginous fungus *Mortierella alpina* expressing heterologous the $\Delta 17$ -desaturase gene under ordinary temperature. *Eur. J. Lipid Sci. Technol.* **2015**, *117*, 1919–1927. <https://doi.org/10.1002/ejlt.201400657>.
159. Mo, B.K.H.; Ando, A.; Nakatsuji, R.; Okuda, T.; Takemoto, Y.; Ikemoto, H.; Kikukawa, H.; Sakamoto, T.; Sakuradani, E.; Ogawa, J. Characterization of $\omega 3$ fatty acid desaturases from oomycetes and their application toward eicosapentaenoic acid production in *Mortierella alpina*. *Biosci. Biotechnol. Biochem.* **2021**, *85*, 1252–1265. <https://doi.org/10.1093/bbb/zbab123>.
160. Tang, X.; Chen, H.; Mei, T.; Ge, C.; Gu, Z.; Zhang, H.; Chen, Y.Q.; Chen, W. Characterization of an omega-3 desaturase from *Phytophthora parasitica* and application for eicosapentaenoic acid production in *Mortierella alpina*. *Front. Microbiol.* **2018**, *9*, 1878. <https://doi.org/10.3389/fmicb.2018.01878>.

161. Huang, M.; Chen, H.; Tang, X.; Lu, H.; Zhao, J.; Zhang, H.; Chen, Y.; Chen, W. Two-stage pH control combined with oxygen-enriched air strategies for the highly efficient production of EPA by *Mortierella alpina* CCFM698 with fed-batch fermentation. *Bioprocess. Biosyst. Eng.* **2020**, *43*, 1725–1733. <https://doi.org/10.1007/s00449-020-02367-9>.
162. Qiu, X.; Hong, H.; Mackenzie, S.L. Identification of a Delta 4 fatty acid desaturase from *Thraustochytrium* sp. involved in the biosynthesis of docosahexanoic acid by heterologous expression in *Saccharomyces cerevisiae* and *Brassica juncea*. *J. Biol. Chem.* **2001**, *276*, 31561–31566. <https://doi.org/10.1074/jbc.M102971200>.
163. Vadivelan, G.; Venkateswaran, G. Production and enhancement of omega-3 fatty acid from *Mortierella alpina* CFR-GV15: Its food and therapeutic application. *Biomed. Res. Int.* **2014**, *2014*, 657414. <https://doi.org/10.1155/2014/657414>.
164. Chang, G.; Wu, J.; Jiang, C.; Tian, G.; Wu, Q.; Chang, M.; Wang, X. The relationship of oxygen uptake rate and k(L)a with rheological properties in high cell density cultivation of docosahexaenoic acid by *Schizochytrium* sp. S31. *Bioresour. Technol.* **2014**, *152*, 234–240. <https://doi.org/10.1016/j.biortech.2013.11.002>.
165. Qu, L.; Ji, X.J.; Ren, L.J.; Nie, Z.K.; Feng, Y.; Wu, W.J.; Ouyang, P.K.; Huang, H. Enhancement of docosahexaenoic acid production by *Schizochytrium* sp. using a two-stage oxygen supply control strategy based on oxygen transfer coefficient. *Lett. Appl. Microbiol.* **2011**, *52*, 22–27. <https://doi.org/10.1111/j.1472-765X.2010.02960.x>.
166. Nandre, R.; Eom, H.; Choi, Y.J.; Zhang, Y.; Ro, H.S. CRISPR/Cas9-mediated gene editing in filamentous fungi, focusing on mushrooms and plant-pathogenic fungi. *Fungal Biol. Rev.* **2025**, *54*, 100446.
167. Kikukawa, H.; Sakuradani, E.; Nakatani, M.; Ando, A.; Okuda, T.; Sakamoto, T.; Ochiai, M.; Shimizu, S.; Ogawa, J. Gene targeting in the oil-producing fungus *Mortierella alpina* 1S-4 and construction of a strain producing a valuable polyunsaturated fatty acid. *Curr. Genet.* **2015**, *61*, 579–589. <https://doi.org/10.1007/s00294-015-0481-2>.
168. Kikukawa, H.; Sakuradani, E.; Ando, A.; Okuda, T.; Ochiai, M.; Shimizu, S.; Ogawa, J. Disruption of lig4 improves gene targeting efficiency in the oleaginous fungus *Mortierella alpina* 1S-4. *J. Biotechnol.* **2015**, *208*, 63–69. <https://doi.org/10.1016/j.jbiotec.2015.05.020>.
169. Vorapreeda, T.; Thammarongtham, C.; Palasak, T.; Srisuk, T.; Jenjaroenpun, P.; Wongsurawat, T.; Nookaew, I.; Laoteng, K. Systematic genome analysis of a novel arachidonic acid-producing strain uncovered unique metabolic traits in the production of acetyl-CoA-derived products in *Mortierella* fungi. *Gene* **2020**, *741*, 144559. <https://doi.org/10.1016/j.gene.2020.144559>.
170. Okuda, T.; Ando, A.; Negoro, H.; Kikukawa, H.; Sakamoto, T.; Sakuradani, E.; Shimizu, S.; Ogawa, J. Omega-3 eicosatetraenoic acid production by molecular breeding of the mutant strain S14 derived from *Mortierella alpina* 1S-4. *J. Biosci. Bioeng.* **2015**, *120*, 299–304. <https://doi.org/10.1016/j.jbiosc.2015.01.014>.
171. Zhang, Y.; Liu, Z.; Xiao, G.; Shi, J.; Liu, B.; Xiao, N.; Sun, Z. Simultaneous DHA and organic selenium production by *Schizochytrium* sp.: A theoretical basis. *Sci. Rep.* **2023**, *13*, 15607. <https://doi.org/10.1038/s41598-023-42900-w>.
172. Li, X.H.; Lu, H.Z.; Yao, J.B.; Zhang, C.; Shi, T.Q.; Huang, H. Recent advances in the application of CRISPR/Cas-based gene editing technology in Filamentous Fungi. *Biotechnol. Adv.* **2025**, *81*, 108561. <https://doi.org/10.1016/j.biotechadv.2025.108561>.
173. Gluth, A.; Trejo, J.B.; Czajka, J.J.; Deng, S.; Qian, W.J.; Yang, B.; Zhang, T. Proteomic insights into the physiology and metabolism of oleaginous yeasts and filamentous fungi. *Front. Microbiol.* **2025**, *16*, 1637123. <https://doi.org/10.3389/fmicb.2025.1637123>.
174. Ye, C.; Xu, N.; Chen, H.; Chen, Y.Q.; Chen, W.; Liu, L. Reconstruction and analysis of a genome-scale metabolic model of the oleaginous fungus *Mortierella alpina*. *BMC Syst. Biol.* **2015**, *9*, 1. <https://doi.org/10.1186/s12918-014-0137-8>.
175. Klempová, T.; Slaný, O.; Šišmiš, M.; Marcinčák, S.; Čertík, M. Dual production of polyunsaturated fatty acids and beta-carotene with *Mucor wosnessenskii* by the process of solid-state fermentation using agro-industrial waste. *J. Biotechnol.* **2020**, *311*, 1–11. <https://doi.org/10.1016/j.jbiotec.2020.02.006>.
176. Xin, F.; Wang, R.; Chang, Y.; Xie, Z.; Zhao, Y.; Zhang, H.; Song, Y. Solid-state fermentation produces greater stearidonic acid levels in genetically engineered *Mucor circinelloides*. *Lett. Appl. Microbiol.* **2022**, *75*, 1617–1627. <https://doi.org/10.1111/lam.13829>.
177. Pullen, R.M.; Decker, S.R.; Subramanian, V.; Adler, M.J.; Tobias, A.V.; Perisin, M.; Sund, C.J.; Servinsky, M.D.; Kozlowski, M.T. Considerations for domestication of novel strains of filamentous fungi. *ACS Synth. Biol.* **2025**, *14*, 343–362. <https://doi.org/10.1021/acssynbio.4c00672>.
178. Alakhras, R.; Bellou, S.; Fotaki, G.; Stephanou, G.; Demopoulos, N.A.; Papanikolaou, S.; Aggelis, G. Fatty acid lithium salts from *Cunninghamella echinulata* have cytotoxic and genotoxic effects on HL-60 human leukemia cells. *Eng. Life Sci.* **2015**, *15*, 243–253. <https://doi.org/10.1002/elsc.201400208>.
179. Kalampounias, G.; Gardeli, C.; Alexis, S.; Anagnostopoulou, E.; Androutopoulou, T.; Dritsas, P.; Aggelis, G.; Papanikolaou, S.; Katsoris, P. Poly-unsaturated fatty acids (pufas) from *Cunninghamella elegans* grown on glycerol induce cell death and increase intracellular reactive oxygen species. *J. Fungi* **2024**, *10*, 130. <https://doi.org/10.3390/jof10020130>.

180. Sayegh, F.; Elazzazy, A.; Bellou, S.; Mohammed, A.; Elkady, A.I.; Baeshen, M.N.; Aggelis, G. Production of polyunsaturated single cell oils possessing antimicrobial and anticancer properties. *Ann. Microbiol.* **2015**, *66*, 937–948. <https://doi.org/10.1007/s13213-015-1176-0>.
181. Kalampounias, G.; Gardeli, C.; Dritsas, P.; Karayannis, D.; Androutsopoulou, T.; Papanikolaou, S.; Aggelis, G.; Katsoris, P. Polyunsaturated fatty acids from *Thamnidium elegans* and *Mortierella alpina* suppress prostate cancer cell proliferation and migration. *Fermentation* **2024**, *10*, 578. <https://doi.org/10.3390/fermentation10110578>.
182. Chan, L.G.; Cohen, J.L.; de Moura Bell, J. Conversion of agricultural streams and food-processing by-products to value-added compounds using filamentous fungi. *Annu. Rev. Food Sci. Technol.* **2018**, *9*, 503–523. <https://doi.org/10.1146/annurev-food-030117-012626>.
183. Nunes, D.D.; Pillay, V.L.; Rensburg, E.V.; Pott, R.W.M. Oleaginous microorganisms as a sustainable oil source with a focus on downstream processing and cost-lowering production strategies: A review. *Bioresour. Technol. Rep.* **2024**, *26*, 10187. <https://doi.org/10.1016/j.biteb.2024.101871>.
184. Hempenius, R.A.; Van Delft, J.M.; Prinsen, M.; Lina, B.A. Preliminary safety assessment of an arachidonic acid-enriched oil derived from *Mortierella alpina*: Summary of toxicological data. *Food Chem. Toxicol.* **1997**, *35*, 573–581. [https://doi.org/10.1016/s0278-6915\(97\)00025-2](https://doi.org/10.1016/s0278-6915(97)00025-2).

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