REVIEW

Open Access

Marine microorganisms: natural factories for polyunsaturated fatty acid production



Li Tian^{1,2†}, Guoxiang Chi^{1,2†}, Sanqian Lin^{1,2}, Xueping Ling^{1,2*} and Ning He^{1,2*}

Abstract

Dietary consumption of omega-3 long-chain polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), is associated with various health benefits. Numerous marine microorganisms, such as bacteria, yeast, microalgae, protists, fungi, and dinoflagellates, produce long-chain PUFAs. These PUFAs are biosynthesized via two pathways: the conventional aerobic fatty acid synthase (FAS) pathway in microalgae and the anaerobic PUFA synthase pathway, primarily in marine bacteria and eukaryotic microalgae. Understanding the mechanisms of long-chain PUFAs (LC-PUFAs) biosynthesis is crucial for developing effective strategies to engineer biosynthesis in both heterologous microbial species and native PUFAs producers. This review highlights recent findings on the biosynthetic mechanisms underlying long-chain PUFAs production, with an emphasis on marine bacteria and microalgae. Additionally, developments in metabolic engineering to enhance long-chain PUFAs production in marine microorganisms are discussed.

Keywords Polyunsaturated fatty acids, Metabolic engineering, Marine microorganism, Biosynthetic pathway

Introduction

Polyunsaturated fatty acids (PUFAs) are essential for the human body and are classified into two types: omega-3 (ω -3) and omega-6 (ω -6) [1]. PUFAs have been shown to benefit hypertension [2], prevent chronic kidney disease [3] and improve lipid metabolism [4]. Additionally, PUFAs can enhance immune function [5] and promote brain and optic nerve development in infants when supplemented during pregnancy [6]. While PUFAs have the

⁺Li Tian and Guoxiang Chi contributed equally to this work.

*Correspondence: Xueping Ling xpling@xmu.edu.cn Ning He

hening@xmu.edu.cn

¹ Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, Xiamen University,

Xiamen 361005, China

² The Key Laboratory for Synthetic Biotechnology of Xiamen City, Xiamen University, Xiamen 361005, China

potential to provide health benefits, further research is necessary to fully understand their effects.

Nature utilizes two biosynthetic pathways for the production of polyunsaturated fatty acids. With their presence in microorganisms dependent on environmental conditions. Aerobic microorganisms use aerobic pathways for synthesizing unsaturated fatty acids. For instance, cyanobacteria, some algae, and plants exclusively utilize aerobic pathways for this purpose. In contrast, certain anaerobic organisms such as E. coli and marine microorganisms that inhabit low-temperature, high-pressure, and anoxic environments only use the anaerobic pathway for unsaturated fatty acid synthesis. Notably, certain bacteria such as Pseudomonas aeruginosa, Tisochrysis lutea, Nannochloropsis [7], Aurantiochytrium SW1 [8] and Thraustochytrids possess the ability to utilize two synthesis pathways simultaneously. These bacteria exhibit unique adaptability and diversity under various environmental conditions. For example, they may need to survive in extreme environments, necessitating the utilization and optimization



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

of their two distinct synthesis pathways to obtain the required unsaturated fatty acids [9, 10].

Traditionally, fish oils have been the main source of PUFAs. The increasing consumer interest in marine species like salmon, known for their rich oil content, has led to intensified fishing efforts. Fish obtain PUFAs by consuming PUFA-rich diets, which results in differences in the fatty acid profile and ratio in their oils. To boost PUFA levels in fish oils, oil-producing microorganisms, such as Schizochytrium sp., are often added [11]. Moreover, to improve the oxidative stability of PUFAs in fish oil and minimize the development of off-flavors, various advanced techniques are applied [12, 13]. However, these methods also contribute to higher production costs. As quality of life and consumption patterns advance, reliance solely on marine fisheries and aquaculture for PUFAs has proven insufficient to meet rising demand. Additionally, growing marine environmental issues highlight the need to diversify PUFAs sourcing methods beyond limited marine resources. Consequently, researchers are increasingly focusing on alternative PUFAs acquisition methods.

In recent years, there has been a significant shift towards using oil accumulating microorganisms like algae and other marine organisms to produce healthbeneficial PUFAs such as EPA and DHA with an ecofriendly and efficient way, marking a new direction for sustainable development. Previous literature has detailed the functionalities of the catalytic domains within fatty acid synthase and PUFA synthase, as well as the mechanisms underlying PUFAs synthesis and the regulation of fatty acid profiles through metabolic engineering modifications [14-18]. The potential of marine microorganisms as a sustainable energy source was discussed, and the adaptability of marine microorganisms to various environmental conditions and high fat content was emphasized. In addition, this review summarizes recent research on unsaturated fat biosynthesis and provides insights into sustainable development and industrial production methods. The mechanism of biosynthesis of long-chain PUFA in marine bacteria and microalgae, as well as advances in metabolic engineering, was comprehensively analyzed to increase PUFA production in marine microorganisms.

Long-chain PUFAs biosynthetic pathway

Long-chain polyunsaturated fatty acids are synthesized via two pathways: the conventional aerobic fatty acid synthase (FAS) pathway and the anaerobic PUFA synthase (polyketide synthase-like, PKS-like) pathway [17, 18].

In the FAS aerobic pathway, specific desaturases and elongases catalyze a sequence of elongation and desaturation steps [19]. Desaturases require oxygen to produce long-chain PUFAs from C16 or C18 saturated fatty acids, which occurs mainly in microalgae, fungi, and plants [20, 21]. Palmitic acid (PA, C16:0) is synthesized using acetyl CoA as a precursor and FAS synthase, with NADPH participating in the process [22, 23]. FAS synthase is a barrelshaped enzyme complex with multiple catalytic domains, including acyl carrier protein (ACP), malonyl palmitoyl transferase (MPT), ketoacyl synthase (KS), ketoacyl reductase (KR), dehydratase (DH), enoyl reductase (ER), and thioesterase (TE) domains [15, 24]. The center of the barrel is divided into two reaction chambers, where catalytic reactions shuttle through the ACP domain [25, 26]. The MPT domain is responsible for loading the starting unit. The process begins with KS domain catalyzing the substrate condensation to form 3-ketoacyl-ACP. Next, KR domain catalyzes the reduction of acyl-ACP to form 3-hydroxyacyl-ACP. The DH domain then catalyzes the dehydration to form 2-trans-enoyl-ACP. Finally, ER domain catalyzes the reduction reaction. This fourstep process (condensation, reduction, dehydration, and reduction) is repeated seven times to produce palmitic acid. Stearic acid (SA, C18:0) is produced through an elongation process by the enzyme [22]. The TE domain catalyzes the formation of free fatty acids. Based on this, oleic acid (OA, C18:1), linoleic acid (LA, C18:1) are synthesized step by step using desaturation enzyme ($\Delta 9$, Δ 12, and Δ 15) [27]. Two desaturase and elongase pathways have been identified for the synthesis of long-chain PUFAs. The conventional $\Delta 6$ pathway utilizes $\Delta 6$ desaturase to form γ -linolenic acid (GLA, C18:3) and stearidonic acid (SDA, C18:4) from LA and ALA, respectively. Then, the $\Delta 6$ elongase extends by 2 carbon atoms to produce dihomo-y-linolenic acid (DGLA, C20:3) and eicosatetraenoic acid (ETA, C20:4). This pathway then converts DGLA to arachidonic acid (ARA, C20:4) and ETA to EPA using $\Delta 5$ desaturase, which can also catalyze EPA formation via omega-3 desaturase [28, 29]. The $\Delta 8$ pathway, mainly found in protist organisms, uses $\Delta 9$ elongase to catalyze the extension of LA and ALA by 2 carbon atoms to yield eicosadienoic acid (EDA, C20:2) and eicosatrienoic acid (ERA, C20:3), respectively, which are then converted by $\Delta 8$ desaturase to DGLA and ETA. The subsequent synthesis steps are identical in the $\Delta 6$ pathway [30]. Figure 1 illustrates the route of PUFAs synthesis by FAS in marine microorganisms.

The FAS pathway requires oxygen, whereas the anaerobic PUFA synthase pathway functions with or without oxygen and is widespread in marine microorganisms, including eukaryotic microalgae and mainly marine bacteria [14, 15, 24]. The PUFA synthase pathway is considered more efficient system than the aerobic pathway because it requires less NADPH to produce longchain PUFAs [24]. Most PUFA synthases from marine microorganisms consist of three or four subunits, each



Fig. 1 Aerobic fatty acid synthase (FAS) pathway in marine microorganisms

producing specific PUFAs, although the multiple catalytic domains in each subunit are very similar [14, 15]. These PUFA synthases are large enzyme complexes with multiple catalytic domains, including acyl carrier protein (ACP), malonyl coenzyme A: ACP transacylase (MAT), ketoacyl synthase (KS), ketoacyl reductase (KR), dehydratase (DH_{PKS} and DH_{FabA}), enoyl reductase (ER), acyltransferase (AT), and chain length factor (CLF) domains [31, 32]. All these domains are involved in the elongation cycle of PUFAs biosynthesis. The biosynthesis of PUFAs begins from acetyl-ACP and proceeds with repeating cycles of four reactions: condensation (KS), ketoreduction (KR), dehydration (DH), and enoylreduction (ER) [15, 24]. The structure and function of biosynthetic domains for ARA, EPA, and DHA synthases have been identified in the marine prokaryotes and microalgal genera, but the detailed biosynthetic machinery remains unclear. Figure 2 is PKS-like synthetic routes for PUFAs in marine microorganisms.

Structure and function of PUFA synthases from marine microorganisms

Fatty acid chain extensions typically contain at least three functional domains, MAT/AT, KS/CLF, and ACP, while KR, DH, and ER act as modifying domains to convert the β -keto groups formed in the condensation step to hydroxyl, olefin, and methylene groups, respectively [15, 22, 33].

Extension of fatty acid chains

The MAT domain is located in the first subunit (*PfaA* or *Subunit A*) of PUFA synthase, which has a high sequence



Fig. 2 Anaerobic PUFA synthase pathway in marine microorganisms

similarity to FabD from E. coli and typical features of α/β hydrolases [34–36]. It is responsible for catalyzing malonyl-CoA, transferring malonyl groups to form malonyl-ACP as an extender for the initiation of fatty acid synthesis [34-36]. Our recent data showed that the reorientation of the MAT-Ser154 C^{β} -O^{γ} bond establishes distinctive proton transfer chains (His153-Ser154 and Asn235-His153-Ser154) for catalysis [37]. Replacement of Gln66 with tyrosine shortens the distance between His153 (N^{ϵ 2}) and Ser154 (O^{γ}), facilitating more rapid proton transfer [37]. In addition, the AT domain located in the second subunit (PfaB or Subunit B) of PUFA synthase usually contains a specific conserved GXSXG motif, which exhibited thioesterase-like activity towards longchain PUFAs thioesters [38, 39]. The Ser96-His220 catalytic dyad can be observed in the AT domain [38, 40].

PUFA synthase typically have multiple KS domains located on different subunits [41, 42]. The elongation of the fatty acid chain depends on an iterative

decarboxylation-Claisen condensation reaction catalyzed by KS_{PfaA} or KS_{PfaB/C}-CLF domains [42]. The two types of KS domains of PUFA synthase have different substrate preference in chain length [14]. The KS_{PfaA} domain accepted mainly short and medium chain substrates, whereas the KS_{PfaB/C}-CLF domain accepted medium and long chain substrates [14, 43]. The previously characterized KS-CLF crystal structure of the Moritella marina (PDB: 6RIW) indicated that the KS contains the conserved triad of one cysteine residue and two histidine residues [42]. The triad involved in catalyzing the hallmark decarboxylative condensation reaction of all KS domains. Notably, the switching of a DHA synthase to an EPA synthase is regulated by specific amino acid residues within the KS-CLF domain [14, 42]. Although it is now evident that the KS domain plays a key role in specifying the chain length of long-chain PUFAs, the precise mechanism of substrate recognition and chain length regulation remains uncertain.

During fatty acid biosynthesis, the acyl group is attached to the conserved serine residue of the ACP domain via the phosphopantetheinyl arm [44]. The ACP domain transfers acyl groups to various functional domains within the PUFA synthase through interactions with the acyl substrates [44, 45]. Through interactions with the different functional domains, the ACP domain is able to direct the acyl substrates to the correct position and ensure the smooth synthesis of the fatty acid chain [44, 45]. Despite the fact that the ACP domains of PUFA synthase can exist in different forms, they are structurally similar [23, 45]. The number of ACP domain may be related to the LC-PUFAs production rate [23, 45].

Modification of fatty acid chains

The catalytic reaction of the KR domain of PUFA synthase reduces 3-ketolipoyl ACP to 3-hydroxylipoyl ACP using NADPH as a cofactor [24, 45]. The domain typically contains a Rossmann fold and a catalytic triad consisting of serine, tyrosine, and leucine residues [46]. The presence of only one KR domain in PUFA synthases suggested that the KR domain may have an even broader acyl substrate specificity [15, 46].

The DH domain possesses a double "hot dog" architecture that catalyzes the dehydration of 3-hydroxylacyl-ACP to 3-enoyl-ACP, thereby introducing a double bond into the fatty acid acyl chain [47, 48]. PUFA synthases typically contain three DH domains, while different types of DH_{PKS} and DH_{FabA} have different substrate preferences in chain length [21, 46]. For an EPA synthase from *Photobacterium profundum*, the DH_{PKS} domain has substrate specificity for 3-hydroxyacyl-ACP with four carbons, whereas the DH_{FabA} domain has selective activity for chain length of six carbons [49-51]. It was confirmed that DH_{PKS} and DH_{FabA} are the key domains for ARA, EPA, and DHA synthesis. The fusion expression of the EPA synthase gene and the ARA synthase gene can adjust the ratio of EPA and ARA [43]. Researchers found that DH domains in Schizochytrium sp. HX-308 and Shewanella sp. BR-2 had 24% and 26% similarities with DH_{FabA} and DH_{PKS} , respectively. The DH_{FabA} "hotdog" linker in Schizochytrium was more flexible than that in Shewanella. Moreover, expressing Schizochytriumderived DH_{FabA} in *E. coli* promoted the accumulation of PUFAs [52].

The ER domain reduces 2-*trans* enoyl-ACPs to saturated acyl-ACPs [49]. One ER is usually present as an individual subunit in the prokaryotic PUFA synthase from marine bacteria, and the ER domain of different prokaryotic PUFA synthases is switchable [49, 53]. However, most PUFA synthases from eukaryotic microorganisms such as *Schizochytrium* sp., *Thraustochytrium* sp., and *Aurantiochytrium* sp., usually comprise two ER

domains that are fused to other domains in two separate subunits [49, 53]. To date, the exact function of the individual ER domains remains to be clarified. Figure 3 depicts gene clusters for the synthesis of polyunsaturated fatty acid synthase, which have been identified in various marine microorganisms.

Diversity and optimization strategies for PUFAs production by marine microorganisms

Biotechnological synthesis of omega-3 polyunsaturated fatty acids, such as DHA and EPA, from marine microorganisms offers a promising strategy for sustainable production [54, 55]. Numerous marine microorganisms, including bacteria, yeast, microalgae, protists, fungi, and dinoflagellates, have been reported as producers of longchain PUFAs [56]. To meet the demands for sustainable and economically viable production, research is being conducted on engineering biosynthetic pathways for unsaturated fatty acids in both heterologous microbial hosts and native unsaturated fatty acids producers [15, 56]. The FAS aerobic pathway require oxygen to produce long-chain PUFAs from C16 or C18 saturated fatty acids, which operates mainly in microalgae, fungi, and plants [20, 21]. Specific desaturases and elongases are required to catalyze a sequence of extension and desaturation steps [20, 21]. In contrast, PUFAs synthesized by the anaerobic PUFA synthase pathway are widespread in marine bacteria (Photobacterium sp., Colwellia sp., Vibrio, Moritella marina, and Shewanella sp.) and eukaryotic microalgae (Schizochytrium sp. and Thraustochytrium sp.) [14, 15, 24]. A summary of the sources of EPA/DHA-producing marine microorganisms is presented in Table 1. And the key domains of PUFA synthases from marine microorganisms exhibited a high degree of homology, despite the fact that they produced different major products [57]. The high conservation of these enzymes indicates they can be efficiently expressed and functional in various hosts. Since these synthetases perform similar roles across species, established methods can be used to optimize or alter them to boost PUFAs production or modify its fatty acid profile. Therefore, these enzymes are ideal for bioengineering strategies aimed at enhancing PUFA output. Utilizing bioengineering to manipulate the unsaturated fat biosynthesis pathway in both heterologous microbial hosts and native marine bacteria is a highly promising approach.

Marine bacteria PUFA synthases

Many marine bacteria synthesize PUFAs through the PUFA synthase system, such as *Shewanella oneidensis*, *Colwellia psychrerythraea*, *Moritella marina*, and *Aureispira marina*. Engineering the PUFA synthase pathway in both heterologous microbial species and native **Prokaryotic PUFA synthases:**



Fig. 3 Gene clusters for the synthesis of PUFA synthase have been identified in various marine microorganisms. KS: ketoacyl synthase; KR: ketoacyl reductase; ER: enoyl reductase; DH: dehydratase; PPTase: phosphopantetheinyl transferase; ACP: acyl carrier protein; MAT: malonyl-CoA transferase; AT: acyltransferase; CLF: chain length factor

marine bacteria is considered a promising alternative. For instance, expressing a prokaryotic PUFA synthase from Shewanella putrefaciens in E. coli resulted in the production of EPA [72]. The co-expression of PUFA synthase along with a phosphopantetheinyl transferase from Moritella marina in E. coli resulted in the production of longchain PUFAs at a concentration of approximately 5 μ g/g on a dry weight basis [73]. Additionally, the expression of a PUFA synthase from Colwellia psychrerythraea in E. coli produced DHA at 2.2 mg per gram of cell dry weight [74]. In addition to E. coli, certain industrial microorganisms may also be employed as chassis. For instance, Lactococcus lactis, Myxococcus xanthus, Pseudomonas putida, and Yarrowia lipolytica are employed as chassis for the heterologous expression of prokaryotic PUFA synthases [15, 75, 76]. However, marine bacteria strains have received less attention in regard for PUFAs production.

Eukaryotic microalgal PUFA synthases

Thraustochytrids, particularly *Schizochytrium* (now *Aurantiochytrium*) have garnered attention for their

capacity to produce high levels of long-chain PUFAs, especially DHA [24]. Additionally, certain species (*Schizochytrium* sp. ATCC PTA-9695, *Schizochytrium* sp. ATCC20888, and *Schizochytrium* sp. MYA1381) accumulate relatively high levels of EPA [21, 35, 77]. Consequently, various strategies have been developed to improve the productivity and economic viability of omega-3 long-chain PUFAs production in *Schizochytrium* and other *Thraustochytrids* [78–83]. These studies has been well discussed in several recent reviews [15, 24, 33, 36]. Here, we will focus on modifying the regulation of the PUFA synthases to improve productivity of omega-3 long-chain PUFAs.

MAT domain is responsible for catalyzing malonyl-CoA, transferring malonyl groups to form malonyl-ACP as an extender for the initiation of fatty acid synthesis [35, 37]. The overexpression of MAT in *Schizochytrium* sp. led to a 10% increase in lipid content and approximately a 24% increase in PUFAs content [35]. Moreover, AT domain exhibited thioesterase-like activity towards long-chain PUFAs thioesters [38, 40]. Recombinant

Microorganism	EPA	DHA	Ref	
Bacteria	Photobacterium profundum Shewanella sp. Photobacterium frigidiphilum Vibrio sp.	Rhodopseudomonas sp. Moritella marina Colwellia psychrerythraea Colwellia marinimaniae Psychromonas sp.	[39, 55, 58–71]	
Fungi	Mortierella alpina Pythium ultimum Mortierella elongata Pythium irregulare Schizochytrium sp. ATCC PTA-9695 Schizochytrium sp. ATCC 20888	Aurantiochytrium sp. Thraustochytrium sp. Schyzochytrium SR21		
Algae	Chlorella minitissima Amphidinium sp. Alexandrium sanguinea Asterionella sp. Chlamydomonas sp. Chlorella ellipsoidea Heterosigma akasiwo Nannochloropsis sp. Nitschia ovali Pavlova gyran Phaedacturum tricornutum Tribonema sp.	Crypthecodinium cohnii microalgae MK 8805 Gyrodinium nelsoni Gonyaulaxpolyedra Amphidinium carteri Amphidinium sp. Alexandrium sanguinea Heterocapsa tricuetra Isochrysis galbana Pavlova gyrans Prorocentrum micans Prorocentrum minmum Scripsiella trochoidea		

Table 1 The sources of EPA/DHA-producing marine microorganisms

Schizochytrium sp. with the AT domain from *Shewanella* sp. replacing its native AT domain showed a 3.7-fold increase in EPA accumulation compared to the wild-type strain [39]. The ACP domain transfers acyl groups to various functional domains within the PUFA synthase through interactions with the acyl substrates [44, 45]. The number of ACP domains may correlate with the long-chain PUFAs production rate [23, 44, 45]. The productivity of EPA increased in parallel with the progressive increase in the number of ACP domains, from 4 to 9. When the number of ACP domains was increased to 8, the productivity increase exceeded 16-fold [44, 45]. The findings indicate that the various PUFA synthase domains may represent promising targets for the enhancement of long-chain PUFAs.

In addition to genetic modification of endogenous genes in native hosts, the heterologous expression of eukaryotic PUFA synthase in oleaginous and industrial microbes has also been employed to enhance the production of long-chain PUFAs. Currently, eukaryotic microalgal PUFA synthases have been expressed in *E. coli*, yeast, and oilseed crop (*Brassica napus* and *Arabidopsis*), with corresponding PUFAs detected in the products. For instance, the MAT domain gene (*ScTIOfabD*) from *Schizochytrium* sp. TIO1101 was heterologously expressed in the yeast *Saccharomyces cerevisiae* [35]. As a result, the engineered yeast exhibited a 16.8% enhancement in biomass production and a 62% increase in fatty

acid accumulation [35]. Additionally, a 2A peptide-based method was used to achieve heterologous expression of the EPA biosynthetic pathway from *Shewanella japonica* in *Aurantiochytrium*, resulting in a fivefold increase in EPA production to 2.7 g/L [77]. Furthermore, PUFA synthase and phosphopantetheinyl transferase from marine microalgae were introduced into the oil-producing plant rapeseed, enriching the fatty acid profile of the oilseed crop and achieving the goal of plant secondary metabolism to produce 3.7% DHA and 0.7% EPA [21]. Table 2 summarizes metabolic engineering strategies for LC-PUFAs biosynthetic pathways in heterologous microbial species and native PUFAs producers.

Marine microalgal fatty acid synthases

Marine microalgae have also been identified as a rich source of long chain PUFAs where fatty acid content accounts for 10–20% of total biomass in some species [56]. PUFAs content in marine microalgae is comparatively higher than that of fresh water microalgal species as marine microalgae produce higher amounts of PUFAs to survive in marine environments. *Nannochloropsis* spp., *Porphyridium cruentum, Phaeodactylum tricornutum* and *Chaetoceros calcitrans* have been reported as the best EPA producing microalgae, while *Isochrysis galbana* and *Cryptecodinium* spp. are more suitable for extraction of DHA [56, 87]. There microalgae produce polyunsaturated fatty acids via the elongase/desaturase

Host species	Genesus	LC-PUFAs	Yields	Ref
Escherichia coli	PUFA synthase gene cluster from She- wanella putrefaciens	EPA	2.1% of dry weight	[71]
Escherichia coli	PUFA synthase from Thraustochytrium	DPA DHA	18% of total fatty acids	[71]
Escherichia coli	PUFA synthase gene cluster from <i>Colwellia</i> psychrerythraea	DHA	2.2 mg/g dry weight	[74]
Yarrowia lipolytica	PUFA synthase from <i>Aetherobacter</i> fasciculatus	DHA	16.8% of total fatty acids	[84]
Escherichia coli	PUFA synthase gene cluster from <i>Moritella marina</i>	DHA	1.6~5.0 ug/g dry weight	[73]
Pseudomonas putida	PUFA synthase from Pseudomonas putida	DHA	1.4 mg/g dry weight	[85]
Schizochytrium sp. ATCC MYA1381	Overexpression of MAT; glucose fed-batch fermentation	dha Epa	Total lipid concentration in broth = 110.5 g/L; DHA concentra- tion = 47.4 g/L; EPA concentration = 1.7 g/L	[35]
Schizochytrium sp. HX-308 (CCTCC M209059)	Replacement with AT gene from She- wanella sp.	DHA EPA	47.2% DHA in total fatty acids; 3.8% EPA in total fatty acids	[39]
Lactococcus lactis	PUFA synthase gene cluster from She- wanella baltica	DHA EPA	0.12 mg/g dry weight 1.35 mg/g	[75]
Schizochytrium sp. ATCC 20888	Increasing the number of ACP domains	dha Epa	A more than 16-fold increase in EPA pro- ductivity and a 1.8-fold increase in DHA productivity	[86]

 Table 2
 Metabolic engineerings of the long-chain PUFAs biosynthetic pathways in heterologous microbial species and native PUFAs producers

pathway. Genetic engineering modifications or optimization of culture conditions can increase the lipid content within microalgae, with lipids accounting for up to 70% of the dry weight of the cell [88]. Generally, to improve the lipid content, most of the modification methods used include increasing the intracellular supply of acetyl-CoA and NADPH, enhancing the photosynthetic intensity of algae, inhibiting the competitive pathway of lipid synthesis, and transcription factor engineering, etc. Compared with wild-type microalgae, the intracellular lipid content of genetically engineered microalgae was greatly increased, and the fatty acid profiles were enriched. This will lay a good foundation for the industrialized production of microalgae [89].

Recent studies have shown that most genes for lengthening and desaturase enzymes in fatty acid synthesis are found in microalgae. This discovery opens the possibility of enhancing polyunsaturated fatty acid production in microalgae through metabolic engineering approaches. In *Nannochloropsis oceanica*, overexpression of Δ 12desaturase was induced by a strong promoter, and the cells were incubated under nitrogen-deficient conditions to increase the proportion of polyunsaturated fatty acids in the fatty acid profile [90]. The overexpression of a non-tandem CCCH-type zinc-finger protein in the *Chlamydomonas reinhardtii* membrane lipids desaturates fatty acids and increases the unsaturated fatty acid content [91]. The combined expression of Malonyl CoAacyl carrier protein transacylase and desaturase 5b in *Phaeodactylum tricornutum* resulted in a significant accumulation of DHA and EPA in the engineered microalgae, which were analyzed by GC–MS in TAG with 36.78 μg/mg and 8.14 μg/mg [92]. The overexpression of Δ 6-desaturase in *Nannochloropsis oceanica* not only enhanced its photosynthetic efficiency, but also resulted in a 1.7-fold increase in lipid content compared with the wild type and an EPA production of up to 62.35 mg/g of cell dry weight [93]. Δ 5-desaturase is a rate-limiting enzyme in the synthesis of polyunsaturated fatty acids. Overexpression of the Δ 5-desaturase PtD5b gene from *Phaeodactylum tricornutum* resulted in a 64% increase in the production of PUFAs, including a 58% increase in the production of EPA [94].

Crypthecodinium cohnii, a microorganism recognized for its high DHA content, has been approved by the Food and Drug Administration for DHA production. To improve strain quality, researchers conducted a fermentation supernatant-based adaptive laboratory evolution to identify strains capable of adapting to the challenging conditions of late-stage fermentation supernatant. This method resulted in a significant 51.79% increase in DHA production [95]. Moreover, the choice of carbon sources for cultivating *Crypthecodinium cohnii* influenced biomass production, with glucose resulting in a biomass yield of 110 g/L in batch culture [96]. The utilization of sodium acetate as a carbon source was found to elevate the percentage of DHA in total fatty acids, attributed to the stimulation of intracellular acetyl-CoA synthesis [97].

Isochrysis sp. has been confirmed to possess the elongation/desaturation pathway, with successful expression of Δ 6-elongase, Δ 5-desaturase, and Δ 12-desaturase in *E*. coli, demonstrating their roles in PUFAs synthesis [98-100]. The efficiency of enzymes involved in PUFAs synthesis varies among different algal sources. For instance, Δ 4-desaturases from *Isochrysis* sp. expression in *Saccha*romyces cerevisiae showed a 34% conversion efficiency for DHA, higher than enzymes from other sources [101]. Researchers have not only validated the genes responsible for the PUFAs-producing pathway but have also optimized the culture conditions of Isochrysis sp. to enhance DHA and EPA levels [102]. They discovered that temperature significantly influences the fatty acid profile, with lower temperatures promoting the accumulation of DHA and EPA. Additionally, increasing nitrogen content in the culture medium and the presence of iron (Fe) can stimulate the expression of Δ 5-desaturase, facilitating EPA accumulation [103, 104].

Microalgae have emerged as a research focus in recent years due to their potential as a sustainable energy source, their environmental adaptability, and their high lipid content. This interest has driven increased exploration into their use in biodiesel production. A combined metabolic engineering approach, utilizing external environmental stress conditions, represents an effective strategy for enhancing microalgal biomass and lipid production. Another promising method for increasing the carbon flux in microalgae is heterotrophic modification, which can be used to construct industrially viable chassis cells. However, only a small proportion of microalgae are currently produced on an industrial scale. Consequently, there is a pressing need to advance the development of

Page 9 of 13

microalgae for future industrial applications. Table 3 provides an overview of methods for enhancing the lipid content of microalgae.

Conclusion and perspective

Microorganisms are pivotal as the primary producers of PUFAs in marine ecosystems. They facilitate the transfer of these essential fatty acids throughout the food chain, making the extraction of PUFAs from marine microorganisms more economically viable than from fish oils [116, 117]. Recently, microorganisms capable of producing PUFAs have been successfully isolated from oceanic environments. These microorganisms are commonly found in extreme conditions like the deep sea, characterized by high pressure and low temperatures, or within the gut flora of specific fish species [118]. Strains such as Thraustochytrids, Shewanella sp. and microalgaes have been identified with high lipid content and a wide range of fatty acids, including DHA and EPA, making them highly suitable for industrial applications. In natural settings, synthesized fatty acid chains typically exist in organisms as triglycerides or phospholipids [119]. Fatty acids are often incorporated into phospholipids during the pre-growth phase of bacterial growth. Several studies indicate a correlation between cell membrane fluidity and the saturation levels of membrane lipids, suggesting that higher lipid unsaturation is linked to enhanced membrane fluidity [120, 121]. With phased temperature control, it was found that low temperature stresses glycolysis pathway and TCA cycle, resulting in a reduced energy supply while pentose phosphate pathway is promoted, increasing NADPH and ATP production, thereby enhancing fatty acid synthesis for cell survival.

Table 3 Methods for increasing the lipid content of microalgae

Methodologies	Microalgae species	Effects	Ref
Regulate $CO_{2'}$ temperature and light	Nannochloropsis sp.	Increase in the EPA content by 3.4-folds	[105]
Regulate nitrate, phosphate, silicate, and temperature	Navicula phyllepta MACC8	Increase in total lipid content by 48%	[106]
Regulate macronutrients (N, P, and S)	Chlamydomonas reinhardtii	Increase in TAG by 1.1- folds	[107]
ACCase overexpression	Chlamydomonas reinhardtii	2.4-fold increase in TAGs	[108]
G6PD overexpression	P. tricornutum	2.7-fold increase in lipid content	[109]
ME overexpression	Chlorella protothecoides	2.8-fold increase in total lipid content	[110]
DGAT2 overexpression	Nannochloropsis oceanica	TAG content was increased to 3.2 $\mu g/mg$ dry weight from 0.7 $\mu g/mg$ dry weight	[111]
DGAT1 overexpression	Nannochloropsis oceanica	TAG accumulation of overexpression line grown up to 0.49 g/L, 47% higher than that of control line	[112]
$\Delta 6$ desaturase overexpression	Mortierella alpina	Resulting in a 26.2-fold increase of EPA yield	[113]
Endogenous $\Delta 5$ desaturase overexpression	Phaeodactylum tricornutum	Resulting in 58% increase of EPA production	[94]
Overexpression of bHLH	Nannochloropsis salina	Under N limitation, FAME productivity of the transformants was 33.2% greater than that of the wild type	[114]
Expression of GmDof4 from soybean	Chlorella ellipsoidea	The lipid content was enhanced by 46.4 to 52.9%, with- out growth limitation	[115]

Additionally, low temperature upregulates the expression of pyruvate carboxylase, which facilitates fatty acid desaturation [122]. This trait is particularly crucial in marine environments, where organisms must withstand osmotic stress and low temperatures, resulting in a higher prevalence of PUFAs in marine microorganisms compared to their freshwater counterparts. While some marine microorganisms can produce fatty acids, their slow growth rate and low oil content make it challenging to meet the demand for PUFAs, leading to increased costs for isolation and purification in downstream processes. Transcriptomics technology can identify crucial genes for fatty acid synthesis. Increasing the C/N ratio in the medium can also boost fatty acid accumulation. And optimizing light intensity, nutrient availability, and temperature, can significantly enhance both growth rates and PUFAs production in marine microalgae. Advanced genetic engineering techniques are being developed to synthesize PUFAs from marine microorganisms. Enhancing the efficiency of anaerobic pathways in model microorganisms with higher oil content through heterologous expression can modify the fatty acid profile. However, this approach is challenging due to the complexity of the PUFA synthase gene cluster and the efficiency of gene editing tools. The rapid advancement of computer technology offers new opportunities for modifying marine microbial PUFAs synthesis, significantly enhancing the fatty acid profile, increasing PUFAs production, and uncovering the synthetic mechanisms of PUFA synthase.

The modification of genetic material in marine microorganisms can potentially impact marine ecosystems. The introduction of genetically modified microorganisms may disrupt the natural ecological balance and affect other biological populations. Ensuring the sustainability and societal approval of marine ecosystems and resources is essential. If these fatty acids are used in nutraceutical formulations, it is crucial to guarantee product safety and transparency to mitigate potential health hazards. Researchers and policymakers must address ethical considerations conscientiously, ensuring that research and development initiatives are sustainable, secure, and socially accountable. Thorough toxicological and nutritional assessments are conducted to verify product safety before commercialization. Implementing specific labeling practices is advisable to minimize the spread of antibiotic resistance. While genetic engineering for the synthesis of LC-PUFAs in marine microorganisms holds significant promise, it also presents complex ethical dilemmas. The safety and societal acceptance of this technology can be maximized through comprehensive scientific risk evaluation, stringent regulatory measures, and active public engagement, fostering sustainable progress and balancing economic, social, and environmental concerns.

Marine microorganisms offer new opportunities to enhance the economic productivity of PUFAs and provide effective solutions to meet the growing market demand for high-quality PUFAs. Advancements in genetic engineering have significantly improved the biosynthesis of long-chain PUFAs, such as DHA and EPA, by leveraging the unique metabolic capabilities of these microorganisms. The ongoing research and development in genetic engineering and metabolic optimization of marine microorganisms promise to meet the increasing global demand for high-quality PUFAs. These innovative approaches provide a sustainable and efficient means of producing essential fatty acids, thereby contributing to improved human health and nutrition. Future studies should continue to explore the potential of marine microorganisms and their genetic manipulation to further enhance PUFAs production and realize their full commercial potential.

Authors' contributions

All authors contributed to the study's conception and design. L.T. and G.X.C. conceived the study, searched the literature and performed data extraction and written the first manuscript. S.Q.L. had the idea for the article, performed the main analysis. G.X.C., X.P.L. and N.H. reviewed the original manuscript and critically revised the work. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (Grant Nos. 32170061 and 31871779).

Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This article ensures that there are no ethical or moral issues.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 14 May 2024 Accepted: 27 August 2024 Published online: 22 October 2024

References

- Sijtsma L, et al. Biotechnological production and applications of the ω-3 polyunsaturated fatty acid docosahexaenoic acid. Appl Microbiol Biotechnol. 2004;64(2):146–53.
- Bercea CI, et al. Omega-3 polyunsaturated fatty acids and hypertension: a review of vasodilatory mechanisms of docosahexaenoic acid and eicosapentaenoic acid. Br J Pharmacol. 2021;178(4):860–77.
- Ong KL, et al. Association of omega 3 polyunsaturated fatty acids with incident chronic kidney disease: pooled analysis of 19 cohorts. Bmj. 2023;380:e072909.

- Yang Y, et al. Effects of different n-6/n-3 polyunsaturated fatty acids ratios on lipid metabolism in patients with hyperlipidemia: a randomized controlled clinical trial. Front Nutr. 2023;10:1166702.
- Byun MJ, et al. Omega-3 polyunsaturated fatty acids modify glucose metabolism in THP-1 monocytes. BioRxiv - Cell Biol. 2024;3:582966.
- 6. Wang Q, et al. The effect of supplementation of long-chain polyunsaturated fatty acids during lactation on neurodevelopmental outcomes of preterm infant from infancy to school age: a systematic review and meta-analysis. Pediatr Neurol. 2016;59:54–61.
- Wang Q, et al. Manipulating fatty-acid profile at unit chain-length resolution in the model industrial oleaginous microalgae *Nannochloropsis*. Metab Eng. 2021;66:157–66.
- Shuib S, et al. Co-existence of type I fatty acid synthase and polyketide synthase metabolons in *Aurantiochytrium* SW1 and their implications for lipid biosynthesis. Biochim Biophys Acta Mol Cell Biol Lipids. 2022;1867(12):159224.
- 9. Cronan JE. Unsaturated fatty acid synthesis in bacteria: Mechanisms and regulation of canonical and remarkably noncanonical pathways. Biochimie. 2024;218:137–51.
- Remize M, et al. A ¹³CO² enrichment experiment to study the synthesis pathways of polyunsaturated fatty acids of the Haptophyte Tisochrysis lutea. Mar Drugs. 2021;20(1):22.
- 11. Leyton, TP, et al. Long-term substitution of fish oil with alternative sources in Atlantic salmon (Salmo salar): Performance, health, and consumer appeal. Aquaculture, 2024;590:741073.
- Kalladathvalappil V, et al. Enhancement of oxidative stability of polyunsaturated fatty acid-rich fish oil: microencapsulation using chitosan-whey protein complex and betalain. Int J Food Sci Technol. 2024;59(4):2286–96.
- 13. Ayeloja A-A, et al. Nutritional quality of fish oil extracted from selected freshwater fish species. Food Chem Adv. 2024;4:4100720.
- 14. Hayashi S, et al. Recent advances in functional analysis of polyunsaturated fatty acid synthases. Curr Opin Chem Biol. 2020;59:30–6.
- 15. Qiu X, et al. Molecular mechanisms for biosynthesis and assembly of nutritionally important very long chain polyunsaturated fatty acids in microorganisms. Prog Lipid Res. 2020;79:101047.
- Nava AA, et al. Module-Based Polyketide Synthase Engineering for de Novo Polyketide Biosynthesis. ACS Synth Biol. 2023;12(11):3148–55.
- Nivina A, et al. Evolution and Diversity of Assembly-Line Polyketide Synthases. Chem Rev. 2019;119(24):12524–47.
- Lin Z, Qu X. Emerging diversity in polyketide synthase. Tetrahedron Lett. 2022;110:154183.
- 19. Winwood RJ. Recent developments in the commercial production of DHA and EPA rich oils from micro-algae. Ocl. 2013;20(6):D604.
- Xue Z, et al. Production of omega-3 eicosapentaenoic acid by metabolic engineering of *Yarrowia lipolytica*. Nat Biotechnol. 2013;31(8):734–40.
- 21. Walsh TA, et al. Canola engineered with a microalgal polyketide synthase-like system produces oil enriched in docosahexaenoic acid. Nat Biotechnol. 2016;34(8):881–7.
- Parsons JB, et al. Bacterial lipids: Metabolism and membrane homeostasis. Prog Lipid Res. 2013;52(3):249–76.
- 23. Du F, et al. Biotechnological production of lipid and terpenoid from thraustochytrids. Biotechnol Adv. 2021;48:107725.
- 24. Chi G, et al. Production of polyunsaturated fatty acids by *Schizochytrium* (*Aurantiochytrium*) spp. Biotechnol Adv. 2022;55:107897.
- 25. Maier T. Re-engineering biofactories. Nat Chem Biol. 2017;13(4):344–5.
- Jenni S, et al. Structure of Fungal Fatty Acid Synthase and Implications for Iterative Substrate Shuttling. Science. 2007;316(5822):254–61.
- Nisha A, et al. Effect of Culture Variables on Mycelial Arachidonic acid Production by *Mortierella alpina*. Food Bioprocess Technol. 2008;4(2):232–40.
- 28. Meesapyodsuk D, et al. The front-end desaturase: structure, function, evolution and biotechnological use. Lipids. 2011;47(3):227–37.
- Lippmeier JC, et al. Characterization of Both Polyunsaturated Fatty Acid Biosynthetic Pathways in *Schizochytrium* sp. Lipids. 2009;44(7):621–30.
- Li M, et al. Isolation of a novel C18-Δ9 polyunsaturated fatty acid specific elongase gene from DHA-producing *Isochrysis galbana* H29 and its use for the reconstitution of the alternative Δ8 pathway in *Saccharomyces cerevisiae*. Biotech Lett. 2011;33(9):1823–30.

- Kaulmann U, et al. Biosynthesis of Polyunsaturated Fatty Acids by Polyketide Synthases. Angew Chem Int Ed. 2002;41(11):1866–9.
- 32. Zhang M, et al. Structural Insights into the Trans-Acting Enoyl Reductase in the Biosynthesis of Long-Chain Polyunsaturated Fatty Acids in *Shewanella piezotolerans*. J Agric Food Chem. 2021;69(7):2316–24.
- Morabito C, et al. The lipid metabolism in thraustochytrids. Prog Lipid Res. 2019;76:101007.
- Cheng R, et al. Cloning and functional analysis of putative malonyl-CoA:acyl-carrier protein transacylase gene from the docosahexaenoic acid-producer *Schizochytrium* sp. TIO1101. World J Microbiol Biotechnol. 2013;29(6):959–67.
- Li Z, et al. Overexpression of Malonyl-CoA: ACP Transacylase in *Schiz-ochytrium* sp. to Improve Polyunsaturated Fatty Acid Production. J Agric Food Chem. 2018;66(66):5382–91.
- Li-Beisson Y, et al. The lipid biochemistry of eukaryotic algae. Prog Lipid Res. 2019;74:31–68.
- Chi G, et al. Computationally Guided Enzymatic Studies on Schizochytrium-Sourced Malonyl-CoA:ACP Transacylase. J Agric Food Chem. 2022;70(43):13922–34.
- Almendáriz-Palacios C, et al. Functional Analysis of an Acyltransferase-Like Domain from Polyunsaturated Fatty Acid Synthase in Thraustochytrium. Microorganisms. 2021;9(3):626.
- Ren L-J, et al. Exploring the function of acyltransferase and domain replacement in order to change the polyunsaturated fatty acid profile of *Schizochytrium* sp. Algal Res. 2018;29:193–201.
- 40. Hayashi S, et al. Off-Loading Mechanism of Products in Polyunsaturated Fatty Acid Synthases. ACS Chem Biol. 2020;15(3):651–6.
- Gemperlein K, et al. Polyunsaturated fatty acid biosynthesis in myxobacteria: different PUFA synthases and their product diversity. Chem Sci. 2014;5(5):1733–41.
- 42. Santín O, et al. Structure and Mechanism of the Ketosynthase-Chain Length Factor Didomain from a Prototypical Polyunsaturated Fatty Acid Synthase. Biochemistry. 2020;59(50):4735–43.
- Hayashi S, et al. Control Mechanism for Carbon-Chain Length in Polyunsaturated Fatty-Acid Synthases. Angew Chem Int Ed. 2019;58(20):6605–10.
- Hayashi S, et al. Enhanced production of polyunsaturated fatty acids by enzyme engineering of tandem acyl carrier proteins. Sci Rep. 2016;6:35441.
- Jiang H, et al. The Role of Tandem Acyl Carrier Protein Domains in Polyunsaturated Fatty Acid Biosynthesis. J Am Chem Soc. 2008;130(20):6336–7.
- 46. Xu W, et al. Structural analysis of protein–protein interactions in type I polyketide synthases. Crit Rev Biochem Mol Biol. 2012;48(2):98–122.
- Tsai, SC, et al. Chapter 2 structural enzymology of polyketide synthases, in complex enzymes in microbial natural product biosynthesis, part b: polyketides, aminocoumarins and carbohydrates. Method Enzymol. 2009;459:17–19.
- Li Y, et al. Functional Characterization of a Dehydratase Domain from the Pikromycin Polyketide Synthase. J Am Chem Soc. 2015;137(22):7003–6.
- Hayashi S, et al. Control Mechanism for cis Double-Bond Formation by Polyunsaturated Fatty-Acid Synthases. Angew Chem Int Ed. 2019;58(8):2326–30.
- Xie X, et al. Functional analysis of the dehydratase domains of a PUFA synthase from *Thraustochytrium* in *Escherichia coli*. Appl Microbiol Biotechnol. 2017;102(2):847–56.
- Xie X, et al. Distinct functions of two FabA-like dehydratase domains of polyunsaturated fatty acid synthase in the biosynthesis of very long-chain polyunsaturated fatty acids. Environ Microbiol. 2020;22(9):3772–83.
- 52. Man Y, et al. Identification dehydratase domains from *Schizochytrium* sp. and *Shewanella* sp. and distinct functions in biosynthesis of fatty acids. Bioprocess Biosyst Eng. 2021;45(1):107–15.
- Bumpus SB, et al. Polyunsaturated Fatty-Acid-Like Trans-Enoyl Reductases Utilized in Polyketide Biosynthesis. J Am Chem Soc. 2008;130(35):11614–6.
- Madore C, et al. Essential omega-3 fatty acids tune microglial phagocytosis of synaptic elements in the mouse developing brain. Nat Commun. 2020;11(1):6133.

- Janssen CIF, et al. Long-chain polyunsaturated fatty acids (LCPUFA) from genesis to senescence: The influence of LCPUFA on neural development, aging, and neurodegeneration. Prog Lipid Res. 2014;53:1–17.
- Dewapriya P, et al. Marine microorganisms: An emerging avenue in modern nutraceuticals and functional foods. Food Res Int. 2014;56:115–25.
- Metz JG, et al. Production of Polyunsaturated Fatty Acids by Polyketide Synthases in Both Prokaryotes and Eukaryotes. Science. 2001;293(5528):290–3.
- Rullán-Lind C, et al. Artificial covalent linkage of bacterial acyl carrier proteins for fatty acid production. Sci Rep. 2019;9(1):16011.
- Chaudhary A, et al. Genomic Insights into Omega-3 Polyunsaturated Fatty Acid Producing Shewanella sp. N2AIL from Fish Gut. Biology. 2022;11(5):632.
- 60. Yadavalli R, et al. Simultaneous production of flavonoids and lipids from *Chlorella vulgaris* and *Chlorella pyrenoidosa*. Biomass Conversion and Biorefinery. 2020;12(3):683–91.
- 61. Samanamud, CA, et al. Selection of strains of *Prorocentrum micans* (Ehrenberg 1834) from Peru based on their lipid potential. Biomass Convers Biorefinery. 2024.
- 62. Concórdio-Reis P, et al. Novel exopolysaccharide produced by the marine dinoflagellate *Heterocapsa* AC210: Production, characterization, and biological properties. Algal Res. 2023;70:103014.
- 63. Thoré ESJ, et al. Microalgae. Curr Biol. 2023;33(3):R91-5.
- 64. Carrasco D, et al. A marine *Chlamydomonas* sp. emerging as an algal model. J Phycol. 2020;57(1):54–69.
- 65. Haq S, et al. Investigating A Multi-Domain Polyketide Synthase in *Amphidinium carterae*. Mar Drugs. 2023;21(8):425.
- Guo X, et al. Identification and characterization of an efficient acyl-CoA: diacylglycerol acyltransferase 1 (DGAT1) gene from the microalga *Chlorella ellipsoidea*. BMC Plant Biol. 2017;17(1):48.
- Kojadinovic-Sirinelli M, et al. Exploring the microbiome of the "star" freshwater diatom Asterionella formosa in a laboratory context. Environ Microbiol. 2018;20(10):3601–15.
- 68. Coleman B, et al. The effect of drying, cell disruption and storage on the sensory properties of *Nannochloropsis* sp. Algal Res. 2023;71:103092.
- Bernaerts TMM, et al. Cell disruption of *Nannochloropsis* sp. improves in vitro bioaccessibility of carotenoids and ω3-LC-PUFA. J Funct Foods. 2020;65:103770.
- Huo S, et al. Bacterial intervention on the growth, nutrient removal and lipid production of filamentous oleaginous microalgae *Tribonema* sp. Algal Res. 2020;52:102088.
- Yazawa, K, Production of eicosapentaenoic acid from marine bacteria. Lipids. 1996;31(1Part2):297-300.
- Yazawa K. Production of eicosapentaenoic acid from marine bacteria. Lipids. 1996;31:S297-300.
- Morita N, et al. Biosynthesis of fatty acids in the docosahexaenoic acidproducing bacterium *Moritella marina* strain MP-I. Biochem Soc Trans. 2000;28(6):943–5.
- Du C, et al. DHA production in Escherichia coli by expressing reconstituted key genes of polyketide synthase pathway from marine bacteria. Plos One. 2016;11(9):e0162861.
- Amiri-Jami M, LaPointe G, Griffiths MW. Engineering of EPA/DHA omega-3 fatty acid production by Lactococcus lactis subsp. cremoris MG1363. Appl Microbiol Biotechnol. 2014;98(7):3071–80.
- Guo P, et al. Deciphering and engineering the polyunsaturated fatty acid synthase pathway from eukaryotic microorganisms. Front Bioeng Biotechnol. 2022;10:1052785.
- Wang S, et al. Optimizing Eicosapentaenoic Acid Production by Grafting a Heterologous Polyketide Synthase Pathway in the Thraustochytrid *Aurantiochytrium*. J Agric Food Chem. 2020;68(40):11253–60.
- Dalmia A, et al. Biochemical characterization of lipid metabolic genes of Aurantiochytrium limacinum. Int J Biol Macromol. 2024;259(Pt 1):129078.
- Yoneda, K, et al. Genetic Modification of *Aurantiochytrium* sp. 18W-13a for Enhancement of Proteolytic Activity by Heterologous Expression of Extracellular Proteases. Mar Biotechnol. 2024.
- Shafaghat Z. et al. Growth dynamics and lipid metabolism of Aurantiochytrium sp.: insights into its potential applications. Aquatic Ecol. 2024;58(3):789–99.

- 81. Zhang H, et al. A phospholipid:diacylglycerol acyltransferase is involved in the regulation of phospholipids homeostasis in oleaginous *Aurantiochytrium* sp. Biotechnol Biofuels Bioprod. 2023;16(1):142.
- Olsen PM, et al. Production of docosahexaenoic acid from spruce sugars using Aurantiochytrium limacinum. Bioresource Technol. 2023;376:128827.
- Prabhakaran P, et al. Uncovering global lipid accumulation routes towards docosahexaenoic acid (DHA) production in *Aurantiochytrium* sp. SW1 using integrative proteomic analysis. Biochim Biophys Acta Mol Cell Biol Lipids. 2023;1868(11):15938.
- Gemperlein K, et al. Polyunsaturated fatty acid production by *Yarrowia lipolytica* employing designed myxobacterial PUFA synthases. Nat Commun. 2019;10(1):4055.
- Gemperlein K, et al. Metabolic engineering of Pseudomonas putida for production of docosahexaenoic acid based on a myxobacterial PUFA synthase. Metab Eng. 2016;33:98–108.
- Hayashi S, et al. Enhanced production of polyunsaturated fatty acids by enzyme engineering of tandem acyl carrier proteins. Sci Rep. 2016;6(1):35441.
- Guedes AC, et al. Fatty acid composition of several wild microalgae and cyanobacteria, with a focus on eicosapentaenoic, docosahexaenoic and α-linolenic acids for eventual dietary uses. Food Res Int. 2011;44(9):2721–9.
- Bellou S, et al. Microalgal lipids biochemistry and biotechnological perspectives. Biotechnol Adv. 2014;32(8):1476–93.
- Sun X-M, et al. Enhancement of lipid accumulation in microalgae by metabolic engineering. Biochim Biophys Acta Mol Cell Biol Lipids. 2019;1864(4):552–66.
- Kaye Y, et al. Metabolic engineering toward enhanced LC-PUFA biosynthesis in Nannochloropsis oceanica : Overexpression of endogenous ∆12 desaturase driven by stress-inducible promoter leads to enhanced deposition of polyunsaturated fatty acids in TAG. Algal Res. 2015;11:387–98.
- Wang R, et al. Non-Tandem CCCH-Type Zinc-Finger Protein CpZF_ CCCH1 Improves Fatty Acid Desaturation and Stress Tolerance in *Chlamydomonas reinhardtii*. J Agric Food Chem. 2023;71(45):17188–201.
- 92. Wang X, et al. Enrichment of Long-Chain Polyunsaturated Fatty Acids by Coordinated Expression of Multiple Metabolic Nodes in the Oleaginous Microalga *Phaeodactylum tricornutum*. J Agric Food Chem. 2017;65(35):7713–20.
- Yang F, et al. Harnessing the Lipogenic Potential of ∆6-Desaturase for Simultaneous Hyperaccumulation of Lipids and Polyunsaturated Fatty Acids in Nannochloropsis oceanica. Front Mar Sci. 2019;6:682.
- Peng K-T, et al. Delta 5 Fatty Acid Desaturase Upregulates the Synthesis of Polyunsaturated Fatty Acids in the Marine Diatom *Phaeodactylum tricornutum*. J Agric Food Chem. 2014;62(35):8773–6.
- 95. Liu L, et al. Rewiring the Metabolic Network to Increase Docosahexaenoic Acid Productivity in *Crypthecodinium cohnii* by Fermentation Supernatant-Based Adaptive Laboratory Evolution. Front Microbiol. 2022;13:824189.
- Dubencovs K, et al. Investigation of *Crypthecodinium cohnii* High-Cell-Density Fed-Batch Cultivations. Fermentation. 2024;10(4):203.
- Li Y, et al. Mechanisms of Sodium-Acetate-Induced DHA Accumulation in a DHA-Producing Microalga, Crypthecodinium sp. SUN. Mar Drugs. 2022;20(8):508.
- Thiyagarajan S, et al. Identification and Functional Characterization of Two Novel Fatty Acid Genes from Marine Microalgae for Eicosapentaenoic Acid Production. Appl Biochem Biotechnol. 2019;190(4):1371–84.
- Thiyagarajan S, et al. Heterologous Production of Polyunsaturated Fatty Acids in *E. coli* Using Δ5-Desaturase Gene from Microalga *Isochrysis* Sp. Appl Biochem Biotechnol. 2020;193(3):869–83.
- Han X, et al. Identification and characterization of a delta-12 fatty acid desaturase gene from marine microalgae *lsochrysis galbana*. Acta Oceanol Sin. 2019;38(2):107–13.
- 101. Shi T, et al. Identification of a novel C22-Δ4-producing docosahexaenoic acid (DHA) specific polyunsaturated fatty acid desaturase gene from *Isochrysis galbana* and its expression in *Saccharomyces cerevisiae*. Biotech Lett. 2012;34(12):2265–74.
- 102. Balakrishnan J, et al. Lowering the culture medium temperature improves the omega-3 fatty acid production in marine microalga *Isochrysis* sp. CASA CC 101. Prep Biochem Biotechnol. 2020;51(5):511–8.

- 103. Jeyakumar B, et al. Nitrogen repletion favors cellular metabolism and improves eicosapentaenoic acid production in the marine microalga *lsochrysis* sp. CASA CC 101. Algal Res. 2020;47:101877.
- Ayothi P, et al. Iron and methyl jasmonate increase high-value PUFA production by elevating the expression of desaturase genes in marine microalga *Isochrysis* sp. J Appl Microbiol. 2022;132(3):2042–53.
- 105. Mitra M, Patidar SK, et al. Integrated process of two stage cultivation of *Nannochloropsis* sp. for nutraceutically valuable eicosapentaenoic acid along with biodiesel. Bioresource Technol. 2015;193:363–9.
- Sabu S, Singh ISB, et al. Improved lipid production in oleaginous brackish diatom *Navicula phyllepta* MACC8 using two-stage cultivation approach. 3 Biotech. 2019;9(12):437.
- 107. Ran W, et al. Storage of starch and lipids in microalgae: Biosynthesis and manipulation by nutrients. Bioresource Technol. 2019;291:121894.
- Rengel R, et al. Overexpression of acetyl-CoA synthetase (ACS) enhances the biosynthesis of neutral lipids and starch in the green microalga Chlamydomonas reinhardtii. Algal Res. 2018;31:183–93.
- 109. Xue J, et al. Glucose-6-phosphate dehydrogenase as a target for highly efficient fatty acid biosynthesis in microalgae by enhancing NADPH supply. Metab Eng. 2017;41:212–21.
- 110. Yan J, et al. Engineering a malic enzyme to enhance lipid accumulation in *Chlorella protothecoides* and direct production of biodiesel from the microalgal biomass. Biomass Bioenerg. 2019;122:298–304.
- 111. Zienkiewicz K, et al. Nannochloropsis, a rich source of diacylglycerol acyltransferases for engineering of triacylglycerol content in different hosts. Biotechnol Biofuels. 2017;10(1):8.
- 112. Wei H, et al. A type-I diacylglycerol acyltransferase modulates triacylglycerol biosynthesis and fatty acid composition in the oleaginous microalga, Nannochloropsis oceanica. Biotechnol Biofuels. 2017;10(1):174.
- Shi H, et al. Application of a delta-6 desaturase with α-linolenic acid preference on eicosapentaenoic acid production in *Mortierella alpina*. Microb Cell Factories. 2016;15(1):117.
- Kang NK, et al. Effects of overexpression of a bHLH transcription factor on biomass and lipid production in Nannochloropsis salina. Biotechnol Biofuels. 2015;8(1):200.
- 115. Zhang J, et al. Modeling and constrained multivariable predictive control for ORC (Organic Rankine Cycle) based waste heat energy conversion systems. Energy. 2014;66:128–38.
- Moi IM, et al. Polyunsaturated fatty acids in marine bacteria and strategies to enhance their production. Appl Microbiol Biotechnol. 2018;102(14):5811–26.
- Gladyshev MI, et al. Production of EPA and DHA in aquatic ecosystems and their transfer to the land. Prostaglandins Other Lipid Mediat. 2013;107:117–26.
- Dailey FE, et al. The Microbiota of Freshwater Fish and Freshwater Niches Contain Omega-3 Fatty Acid-Producing *Shewanella* Species. Appl Environ Microbiol. 2016;82(1):218–31.
- 119. Burri L, Hoem N, et al. Marine omega-3 phospholipids: metabolism and biological activities. Int J Mol Sci. 2012;13(11):15401–19.
- 120. Mondal D, Malik S, et al. Modulation of membrane fluidity to control interfacial water structure and dynamics in saturated and unsaturated phospholipid vesicles. Langmuir. 2020;36(41):12423–34.
- 121. Henderson CM, Block DE. Examining the role of membrane lipid composition in determining the ethanol tolerance of *Saccharomyces cerevisiae*. Appl Environ Microbiol. 2014;80(10):2966–72.
- 122. Song Y, et al. Comparative transcriptomic and lipidomic analyses indicate that cold stress enhanced the production of the long C18–C22 polyunsaturated fatty acids in *Aurantiochytrium* sp. Front Microbiol. 2022;13:915773.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.