Archives of Microbiology (2023) 205:321
https://doi.org/10.1007/s00203-023-03666-x

MINI REVIEW

Microbial production of docosahexaenoic acid (DHA): biosynthetic pathways, physical parameter optimization, and health benefits

Naaz Abbas[1] · Sana Riaz[1] · Sania Mazhar[1] · Ramsha Essa[1] · Maria Maryam[1] · Yasar Saleem[1] · Quratulain Syed[1] · Ishrat Perveen[1] · Bakhtawar Bukhari[1] · Saira Ashfaq[1] · Syed Hussain Imam Abidi[1]

Received: 4 July 2023 / Revised: 6 August 2023 / Accepted: 18 August 2023 / Published online: 29 August 2023

Abstract

Omega-3 fatty acids, including docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and α-linolenic acid (ALA), are essential polyunsaturated fatty acids with diverse health benefits. The limited conversion of dietary DHA necessitates its consumption as food supplements. Omega-3 fatty acids possess anti-arrhythmic and anti-inflammatory capabilities, contributing to cardiovascular health. Additionally, DHA consumption is linked to improved vision, brain, and memory development. Furthermore, omega-3 fatty acids offer protection against various health conditions, such as celiac disease, Alzheimer's, hypertension, thrombosis, heart diseases, depression, diabetes, and certain cancers. Fish oil from pelagic cold-water fish remains the primary source of omega-3 fatty acids, but the global population burden creates a demand–supply gap. Thus, researchers have explored alternative sources, including microbial systems, for omega-3 production. Microbial sources, particularly oleaginous actinomycetes, microalgae like *Nannochloropsis* and among microbial systems, *Thraustochytrids* stand out as they can store up to 50% of their dry weight in lipids. The microbial production of omega-3 fatty acids is a potential solution to meet the global demand, as these microorganisms can utilize various carbon sources, including organic waste. The biosynthesis of omega-3 fatty acids involves both aerobic and anaerobic pathways, with bacterial polyketide and PKS-like PUFA synthase as essential enzymatic complexes. Optimization of physicochemical parameters, such as carbon and nitrogen sources, pH, temperature, and salinity, plays a crucial role in maximizing DHA production in microbial systems. Overall, microbial sources hold significant promise in meeting the global demand for omega-3 fatty acids, offering an efficient and sustainable solution for enhancing human health.

Keywords Docosahexaenoic acid (DHA) · Biosynthetic pathways · Physical parameter · Health benefits

Introduction

Omega-3 fatty acids belong to the family of polyunsaturated fatty acids with significant types, including docosahexaenoic (DHA), eicosapentaenoic (EPA, and α-linolenic acid (ALA). The DHA is a vital nutrient that cannot be synthesized by the body but has valuable health benefits in the growth and development of the human body. The conversion of dietary DHA in the human body is gender dependent, and desaturation and elongation of carbon chains may result in the transformation of ALA into DHA (Sheikh et al. 2019). In contrast, men convert only 12% of the ALA they consume into EPA, DPA, and DHA, while females convert 21% of the ALA into EPA and DHA (Burns-Whitmore et al. 2019). The limited conversion of dietary DHA is insufficient to meet the human body's daily dietary demands and needs to be consumed as food supplements. Omega-3 fatty acids possess anti-arrhythmic and anti-inflammatory capabilities and help maintain cardiovascular health (de Abreu Silva et al. 2021). The consumption of DHA improves vision, brain, and memory development in infants and adults.

Moreover, omega-3 fatty acids aid in limiting the symptoms associated with celiac disease, Alzheimer's, hypertension, thrombosis, heart diseases, depression, diabetes, and several cancers (Hashimoto et al. 2015). Fish oil from pelagic cold-water fish species like tuna and salmon is the

Communicated by Yusuf Akhter.

✉ Sana Riaz
sanariazpk@gmail.com

[1] Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex Ferozepur Road, Lahore, Pakistan

primary source of omega-3 fatty acids. Approximately one million metric tons of fish oil are produced worldwide annually. The omega-3 fatty acids from this fish oil are currently used in medicines, foods, and the nutraceutical industry (Karageorgou et al. 2023). However, the global population burden creates a gap between the production and consumption of nutraceutical oils due to the rising demand for fish and fish oil containing omega-3 fatty acids (Turchini et al. 2009). Different manufacturing strategies and sources of omega-3 production are explored to meet the world's reliance on omega-3. Traditional methods of omega-3 production are more laborious and offer various technical difficulties and limitations.

Interestingly, omega-3 fatty acids obtained from microbial systems have an identical composition to those found in plants and animals, such as organic waste (Alfio et al. 2021). Several microbial systems have been studied for the industrial and commercial scale production of omega-3 oil, which includes oleaginous actinomycetes, microalgae for omega-3 production *Nannochloropsis* and *tricornutum* capabilities (Cui et al. 2021), Fig. 1. Regarding categorization, *Thraustochytrids* are classified as algae and fungi. *Thraustochytrids* may store up to 50% of the entire dry weight in lipids. Health professional associations recommend the daily consumption of 500 mg of omega-3 oils, which equates to 1.3 million metric tons of human consumption every year (Papanikolaou and Fulgoni III 2019). When considering the worldwide regulatory authorities' recommendations for daily DHA intake, the consumption needs to reach 500 mg/day (Mun et al. 2019). The average recommended consumption of omega is only followed in coastline countries like Japan and some Scandinavian countries with diets rich in marine species than the rest of the world (Wang et al. 2022).

Health benefits of DHA

Omega-3 fatty acids play a pivotal role as an anti-arrhythmic, anti-inflammatory, anti-thrombotic, anti-atherosclerotic, anti-fibrotic, and endothelial relaxant which aid in preventing cardiovascular diseases. Moreover, it can prevent or treat atherosclerosis, hypertriglyceridemia, high blood pressure, rheumatoid arthritis, asthma, lupus erythematosus, diabetes, migraine, nephritis, and psoriasis (Siriwardhana et al. 2012). PUFAs have become a crucial part of the maternal diet due to their benefits on fetal development. Pregnant or lactating women should eat 8–12 ounces of seafood per week, 300–900 mg DHA + EPA per day

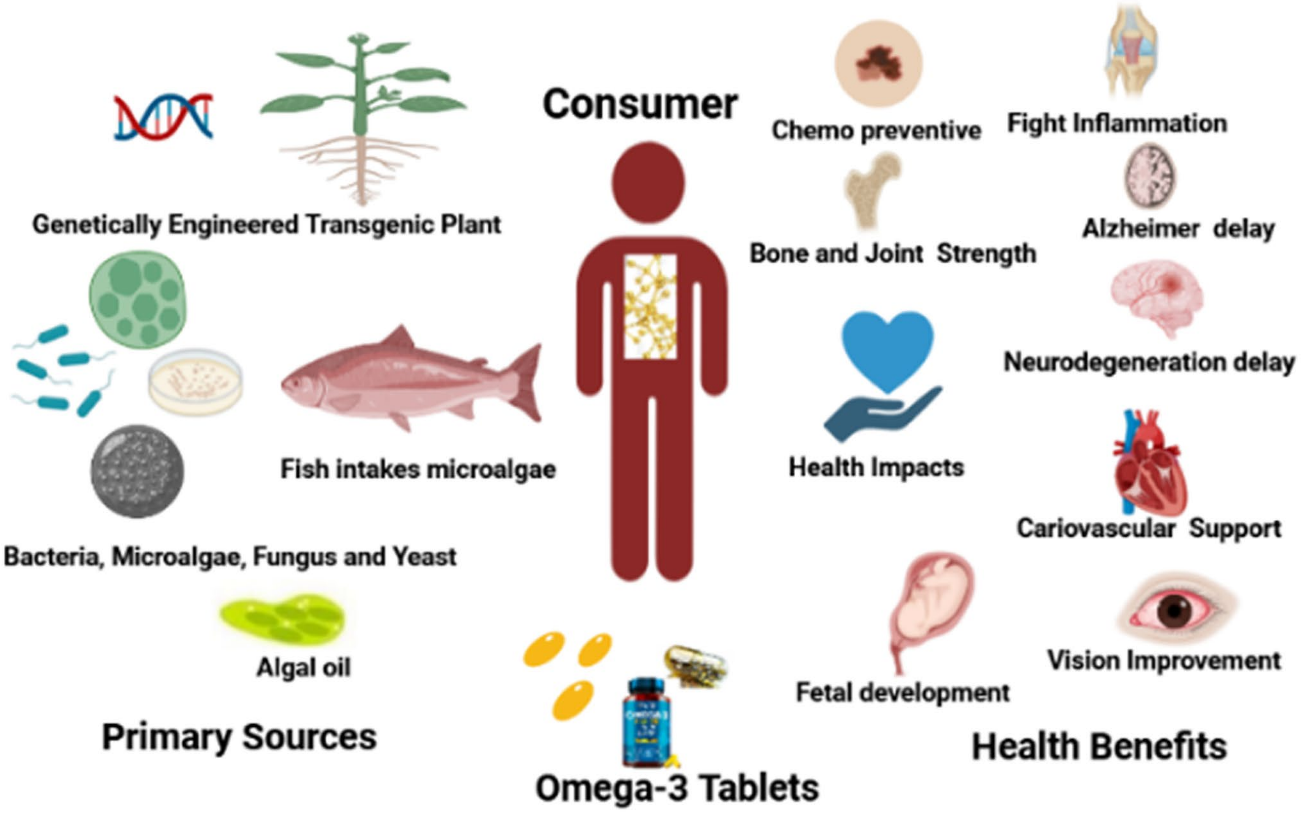

Fig. 1 Primary sources of omega-3 polyunsaturated fatty acids (PUFAs) and health benefits for consumers

(Horrocks and Yeo 1999). This helps in the development of the fetal brain and eyes. DHA and EPA also prevent preterm birth, widely known as a severe cause of infant mortality. Circulatory system disorders are linked with plaques (lipid or calcium) building up in the arteries, which may tend to cause atherosclerosis and blood clots.

Similarly, myocarditis (heart muscle inflammation) accelerates plaque formation in the arteries, creates blood clots, and may result in cardiovascular events (Zhang et al. 2019). Decreasing inflammatory cytokine (*i.e.,* Interleukins IL-6, IL-1b) and the production of tumor necrosis factor (TNF) in such a way that ameliorates its protective role and deteriorates its harmful effects. The high concentration of PUFAs in synaptic neuronal membranes is crucial in neurodegenerative diseases. This modifies vascular and immunological systems, which affects the CNS (Li et al. 2021). PUFAs are essential for embryonic cell growth and neurological development. Research has linked apoptosis to low PUFAs. DHA promotes brain growth, especially in the newborn brain, and protects against apoptotic cell death when consumed during pregnancy (Li et al. 2021). It is a mental health problem characterized by a lower interest in everyday activities or poor performance at work, which is frequently expressed as rage, a sense of loss and sorrow. Hypertension is a rise in blood pressure within the arteries because of enhanced insulin resistance, endothelial dysfunction, and peripheral vascular resistance. Alzheimer's disease (AD) is a common type of dementia, especially in older adults (Liu et al. 2022). Plasmogens, a neuronal lipid, and DHA, which function as neurotransmitters containing vesicles, are both found in synaptosomes and are essential for efficient neurotransmission. Lower levels of plasmogen and DHA are observed in the hippocampus of people diagnosed with Alzheimer's, which has been found to interrupt the neurotransmission process (Chen et al. 2022a). Skin protection, homeostasis, and omega-3 FAs (notably EPA and DHA) are well synchronized. Several skin-related disorders, such as skin cancer, photoaging, allergies, and dermatitis, are improved. Fish oil PUFAs may reduce cutaneous inflammation and improve wound healing by regulating cytokine activity. The previous research, thus, highlights the crucial role of polyunsaturated fatty acids as a therapeutic approach (Ferreira et al. 2022). According to studies, co-microencapsulating probiotic strains with omega-3-rich tuna oil help to maintain high levels of omega-3 FAs in the digestive system, which increases microbial survival and adhesion to the intestines (Zhou and Wei 2023). The genetic engineering of these organisms holds immense potential to enable the more affordable manufacturing of customized oils. Consequently, it will be pertinent for researchers from various fields to investigate the further potential of marine microorganisms for biotechnological applications shortly.

Natural sources of omega-3 fatty acid production

DHA are abundant in fish-derived lipids, and their quantity varies by species and location. Marine fish has abundant DHA, which grazes primarily on zooplankton, a good source of omega-3 fatty acids (Simonato et al. 2023). Recent research has shown the remarkable capability of *Camelina sativa*, which has excellent oil content with more than 35% PUFAs like DHA and EPA at amounts similar to that found in fish oil, thanks to genetic modification (Betancor et al. 2021). Genetically modified plants are a source of PUFAs; however, their DHA levels are often low than those of oleaginous microbes (West et al. 2021). In seeds, transgenic plants generated up to 15% eicosapentaenoic and up to 1.5% DHA; studies have been conducted about the omega-3 production pathway from transgenic plants, ranging from diverse host systems to oilseed plants (Petrie et al. 2020).

Microbial production of omega-3 fatty acid

Despite the challenges, efforts to find new, sustainable sources for synthesizing Omega-3 fatty acids are ongoing. Table 1 presents data on genetically modified microbes and plant for enhanced production of DHA. The biomass titer, total fatty acid (TFA) content, and lipid content of various microbes are involved in the production of omega-3 fatty acids. *Schizochytrium* sp. stands out after overexpression of ATP citrate lyase (ACL), acetyl CoA-carboxylase (ACC), as one of the top-performing microorganisms, achieving biomass titers ranging from 14.0 g/L to 47.4 g/L with TFA content ranging from 42.9 g/L to 54.5 g/L and lipid content from 26.0% to 30.5% (Han et al. 2020) (Li et al. 2018; Guo et al. 2018; Guo et al. 2018; Sun et al. 2018). In *P. tricornutum*, the expression of Δ5-desaturase and Δ6-desaturase gene resulted in boosting the DHA production to many folds. (Hamilton et al. 2014). Similarly, *Aurantiochytrium* sp. demonstrates promising results with biomass titers of 4.8 g/L to 12.5 g/L, TFA content of 23.9 g/L to 52.5 g/L, and lipid content of 13.2% to 28.9% (Heggeset et al. 2019; Ma et al. 2015; Nazir et al. 2018).*Crypthecodinium cohnii* also exhibits potential with biomass titers from 2.7 g/L to 13.1 g/L, TFA content of 24.2 g/L to 54.0 g/L, and lipid content of 13.5% to 33.0% (Diao et al. 2018; Diao et al. 2019; Liu et al. 2018). Notably, *Myxobacteria* spp. and *Pseudomonas putida* have lower TFA content but still play a role in the production of omega-3 fatty acids. These findings emphasize the diversity of microbial sources and their capabilities in the

Table 1 Microbial synthesis of omega-3 PUFA through genetic engineering for enhancing DHA production

Microbe	Biomass titer	TFA content	Lipid content	References
Phaeodactylum tricornutum	–	–	10.4%	(Hamilton et al. 2014)
Schizochytrium sp.	47.4 g/L	42.9 g/L	26.0%	(Li et al. 2018)
	39.2 g/L	42.9 g/L	30.5%	(Guo et al. 2018)
	38.3 g/L	54.5 g/L	30.1%	(Guo et al. 2018)
	38.1 g/L	53.3 g/L	30.0%	(Sun et al. 2018)
	14.0 g/L	50.9 g/L	26.7%	(Zhao et al. 2018)
	–	–	37.9%	(Han et al. 2020)
Aurantiochytrium sp.	12.5 g/L	23.9 g/L	13.2%	(Heggeset et al. 2019)
	10.0 g/L	52.5 g/L	28.9%	(Ma et al. 2015)
	4.8 g/L	52.0 g/L	25.0%	(Nazir et al. 2018)
Crypthecodinium cohnii	13.1 g/L	52.4 g/L	26.2%	(Diao et al. 2018)
	9.6 g/L	24.2 g/L	13.5%	(Liu et al. 2018)
	2.7 g/L	54.0 g/L	33.0%	(Diao et al. 2019)
Myxobacteria sp.	–	11.9 g/L	–	(Gemperlein et al. 2019)
Yarrowia lipolytica	3.50 g/L	16.8 g/L	1.7%	(Garcia et al. 2016)
Pseudomonas putida	3.0 g/L	–	–	(Gemperlein et al. 2016)
Escherichia coli	20.0 g/L	31.7 g/L	–	(Giner–Robles et al. 2018)
	20.0 g/L	7.0 g/L	–	(Peng et al. 2016)
Thraustochytrium aureum and *M. alpine*	–	–	5.6%	(Wang and Pan 2019)
Aetherobacter fasciculatus and *Minicystis rosea*	30.0 g/L	–	16.8%	(Gemperlein et al. 2019)
Brassica napus	–	–	9.7%	(Petrie et al. 2020)

production of omega-3 fatty acids, providing promising alternatives to meet the global demand for these essential nutrients (Gemperlein et al. 2019; Gemperlein et al. 2016).

Algal production

Photosynthetic organisms utilize energy produced in cell division, enhancing bio productivity and not requiring a large surface area for growth. In contrast, microalgae grow in a variety of ways, including phototrophic (using carbon dioxide as a carbon source and sunlight as an energy source), heterotrophic (using organic carbon sources), and mycotrophic (using both organic and inorganic–organic carbon as substrates), which involves both organic and inorganic carbon sources to carry out photosynthesis (Malik et al. 2023). Various parameters influence the microalgae's cellular development rate, including lipid accumulation, dissolved oxygen levels, light intensity, pH, temperature, nutrient concentration, and carbon dioxide levels (Chandrasekhar et al. 2022). The synthesis of TAGs and the assembly of glycerol lipids occur in the microalgae through a series of enzymatic reactions in the endoplasmic reticulum and chloroplasts, respectively (Xu et al. 2020). The synthesis of significant amounts of lipids, specifically neutral fats in the form of TAGs/oil bodies, which may be used as carbon and energy storage sources, is thought to be triggered by physical, chemical, and environmental stresses (Xu 2022). The *Dinoflagellate Crypthecodinium cohnii* and the *Thraustochytrids* (*Schizochytrium, Thraustochytrium,* and *Aurantiochytrium*) are notable heterotrophic organisms that collect substantial amounts of DHA (Bagul and Annapurna 2021a). Despite being categorized as microalgae, these bacteria no longer possess the capacity for photosynthesis and now live in the water as saprophytes that aid in the breakdown of organic debris.

Microalgae; *Aurantiochytrium* and *Schizochytrium*

The practical cell factories for DHA generation are *Schizochytrium spp*. For more than 25 years, unique strains have been employed in the commercial manufacture of meals and DHA (Zhong et al. 2023). The bacteria had anaerobic and aerobic PUFAs, but lacked D12-desaturase, implying that DHA is produced anaerobically. Recently, it was discovered the effectiveness of oxygen in DHA production. When ascorbic acid is added as an antioxidant, intracellular ROS levels are decreased, and DHA titer is increased to 38.3 g/L (Ortiz-Sanchez et al. 2023). The mutant ALE-TF30 was created using adaptive laboratory evolution. It accumulated 38.1 g/L of DHA, 57% more than the parent strain, and had lower lipid peroxidation (Jovanovic et al. 2021). The quantity of reactive oxygen species (ROS) has decreased due to superoxide dismutase's overexpression. Recent bioprocess investigations have demonstrated that media have been improved, and DHA synthesis at a scale of 7000L has been accomplished with 14 DHA g/L created via room temperature plasma mutagenesis and atmosphere (coupled with susceptibility against malonic acid and zeocin) (Jovanovic et al. 2021)(Du et al. 2021). The internal levels of acetyl-CoA and NADPH necessary for PUFA biosynthesis were increased by heterologous production of C16/18 FAs elongase and malic enzymes. However, to meet the dietary recommendations for EPA and DHA, recent advances have focused on increasing the amount of these fatty acids (Tocher et al. 2019). A fascinating reconstruction of the PUFA PKS was made by substituting a homologue from the EPA-producing bacteria *Shewanella sp.* for the native acyltransferase (AT) domain; the lipid composition was significantly modified (Ogawa et al. 2020). EPA and DHA up to 2.3 and 28.8 g/L were then produced using these homologues. Malonyl-CoA ACP transacylase overexpression also changed the carbon flow to PUFA synthesis.

Crypthecodinium cohnii

DHA is solely present in the lipids of *C. ch*ronic, while some evolved strains were also discovered by molecular-based ALE. Reportedly, DHA production has increased to 7.8 g/L by increasing oxygen availability and optimizing nitrogen feeding (Zhang et al. 2023).

Phaeodactylum tricornutum

The *P. tricornutum,* a photosynthetic diatom, cumulates about 36% EPA in its total fatty acids. The glucose transporter was introduced by advancing tropic growth on glucose in dark environments using cultures without light (Archer et al. 2019). Moreover, a mutant strain accumulating 23.6% DHA and 36.5% EPA has been developed using a combination of glucose transporter and D5 elongase. Recently, it has been studied that overexpression of endogenous D6 denaturize and native gene 1-acyl-sn glycerol-3-phosphate improved the selectivity of EPA and primary metabolism, respectively. Furthermore, *P. tricornutum* was modified in the fish industry to produce dual types of phytases along with polyunsaturated fatty acids. One such example is the development of a strain which produces 40,000 phytases and EPA, DPA, and DHA with high percentages of total fatty acids (Branco-Vieira et al. 2020). These developments provide a promising future for enhancing the nutritional value of vegetables for aquacultures.

Fungal and yeast production

Mortierella alpina, *Mucor circinelloides*, and *Yarrowia lipolytica* are dimorphic fungi and yeast that benefit from built-in protection against the hazardous side effects of PUFA production and inherently efficient lipid metabolism (Wang et al. 2023). Yeasts are another type of microorganism that may be used to make nutraceutical PUFA. Yeasts have a fast growth rate and do not require light to thrive, unlike microalgae (Kothri et al. 2020). As a result, they may be scaled up to industrial manufacturing levels. Furthermore, because their lipid composition is influenced by the carbon source used in growing, they may be modified to produce specific lipid profiles. Researchers discovered that just two fungus cultures, *Aspergillus niger* and *Trichoderma spp.* could produce significant amounts of EPA and DHA (Nopparatmaitree et al. 2022).

Yarrowia lipolytica

In recent years, Y. lipolytica can grow in hydrophobic environments and accumulate significant amounts of lipids with unsaturated fatty acid content; this potential has been effectively harnessed. The commercialization of strains that generates EPA was a significant milestone for this purpose; various combinations of heterologous enzymes from various native producers that belong to the alternative aerobic D8-EPA were introduced and assessed. As a result of the research, EPA accounted for above 56.6% of TFAs and 15% of cell dry weight, while saturated fatty acids were restricted below 5% (Eltanahy and Shehatta 2022). By eliminating the PEX10 gene and enhancing the fermentation process, peroxisome biogenesis was suppressed, and as a result, 25.0% of EPA was synthesized (Penno et al. 2020). Further research demonstrated yeast's incredible capacity to synthesize various PUFAs for this purpose. Additionally, the bifunctional D12/D15-desaturase and low-temperature fermentation formed about 1.4 g/L ALA. Recent studies have revealed that genetically altered *Y. lipolytica* can produce DHA from scratch using a PKS-like PUFA synthase (Chi et al. 2022).

PUFA accumulation through *Saccharomyces cerevisiae*

Monounsaturated and saturated fatty acids are produced from *S. cerevisiae,* but no polyunsaturated fatty acids (PUFAs) have been reported to produce from it. Recent research has demonstrated that recombinant *S. cerevisiae* can produce LA, ALA, and EDA, although the production percentage is much lower than other microorganisms (Dawood et al. 2021). When *S. cerevisiae* expresses PUFAs, it becomes intoxicated, displaying caspase-mediated cell death, damaged lipid peroxides, and proteins. Significant engineering appears necessary to produce high-level PUFA from the yeast (Jovanovic et al. 2021).

Bacterial production

Compared to microalgae and fungi, bacteria are considered ineffective EFA producers. Certain barophilic and psychrophilic organisms present in the ocean's depths, such as members of the *Shewanella* genus, are significant PUFA producers. *Acinetobacter calcoaceticus, Bacillus acidophilus, Arthrobacter sp., and Rhodococcus opacus* are other bacteria that generate PUFA. BRI 35 (*Coccoid Actino*bacteria, Gram-positive, *Micrococcaceae*) was isolated from a sample of Antarctic seawater (Alvarez et al. 2021). Their ability to utilize a range of substrates throughout the production process, including organic substrates, *i.e.,* by-products and wastes, is one of the benefits that microbial PUFAs provide over fish oils (Fu et al. 2021).

Biosynthesis of omega-3 fatty acids

A multi-enzyme complex catalysis, the bacterial polyketide method of DHA biosynthesis, is regulated by proteins expressed by five (5) PFA genes which include pfaB, pfaA, pfaC, pfaE, and pfaD (Chaudhary et al. 2022). Numerous cloning studies using these genes have been conducted; the most well-known was carried out in the *Shewanella sp.* strain SCRC-27. Because of their high degree of unsaturation, PUFAs are quickly oxidized. Reactive oxygen species (ROS), produced during peroxidation, that induce significant oxidative damage to the produced cells, diminish product titer, making peroxidation undesirable. The aerobic PUFA biosynthesis route is found in mammals, plants, and eukaryotic microbes (at least in part) (Sun 2020), dimorphic fungi, marine microalgae, *i.e., dinoflagellates***,** heterotrophic protists and photosynthetic haptophytes make up the final category (Wang et al. 2020). The traditional fatty acid (FA) synthesis machinery creates a saturated acyl intermediate, which is then extended to 16 carbon fatty acids, including palmitic acid (PA) and polyunsaturated fatty acids (PUFAs) Fig. 2 (Dietzen et al. 2022).

The discovery of a particular anaerobic route for bacterial PUFA production and the cloning of EPA biosynthetic genes were considered significant advancements in PUFA research (Qiu et al. 2020). The aerobic and anaerobic pathways are

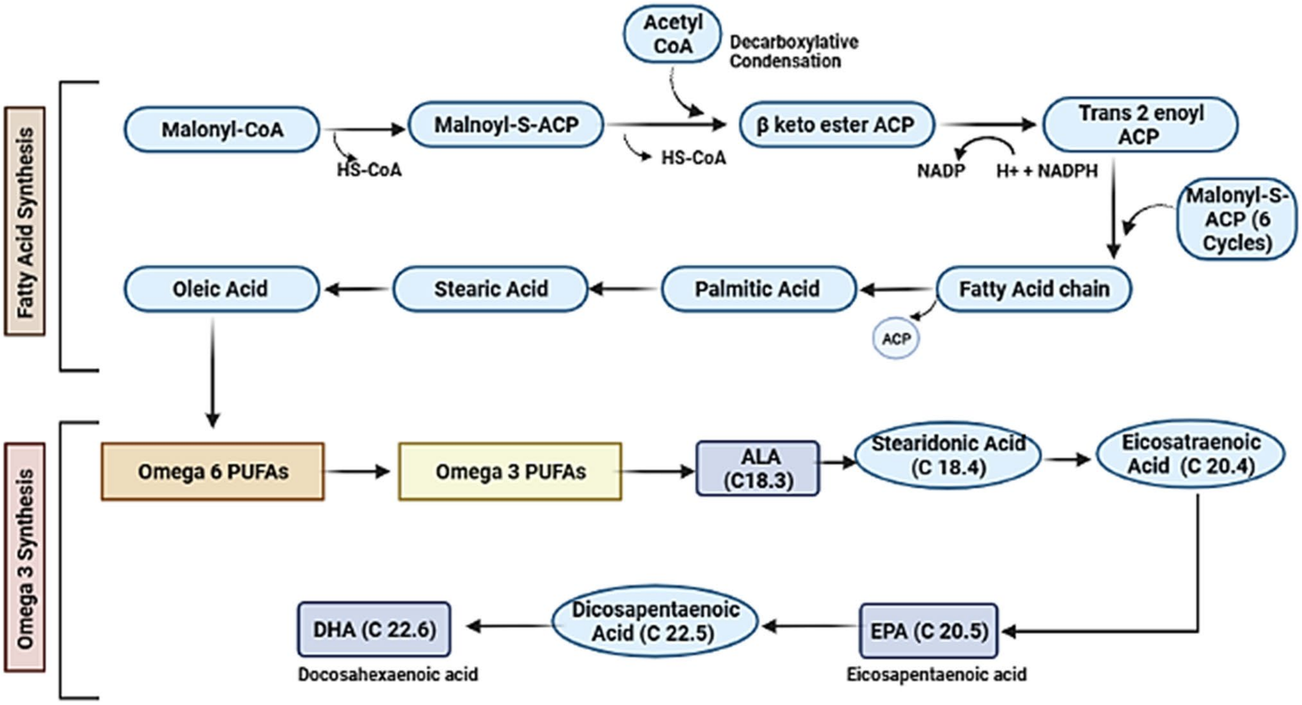

Fig. 2 The aerobic pathway for the production of PUFAs is intricately incorporated into the fatty acid synthesis (FAS) machinery within animals, certain plants, and eukaryotic microbes

different. It is not linked to FAS and instead synthesizes PUFAs by Lenovo from scratch using an enzyme complex which resembles polyketide synthase (PKS)-PUFA synthase (Řezanka et al. 2020). The mechanism comprises repeated cycles of condensation and dehydration followed by keto-reduction and length regulation by the chain length factor (Fig. 3). In unicellular microalgae and bacteria, anaerobic PUFA production has been found. It takes almost half of NAD (P) H as in the aerobic pathway, making it appealing for PUFA overproduction (Deeba et al. 2020).

Purification methods

Omega-3 fatty acids from natural sources have been concentrated and purified using a variety of chromatography processes. This has been accomplished using high-performance liquid chromatography, silver resin chromatography, and supercritical fluid chromatography. For instance, using silica gel columns loaded with silver nitrate, researchers were able to separate pure DHA and EPA from fish oils. The procedure includes saponification, urea treatment, methyl ester conversion, and a final washing step with mobile phases. Depending on the precise technique utilized, these chromatographic techniques produced high-purity omega-3 products with various EPA/DHA ratios, offering a variety of recovery percentages (Perretti et al. 2007a, b). Utilizing the various fatty acids' different melting points, low-temperature fractional crystallization is a simple process for producing refined omega-3 fatty acids. To get pure fractions with particular omega-3 fatty acids, including EPA and DHA, numerous crystallization and separation operations are necessary when the combination comprises many different types of fatty acids (Shahidi 2006)**.** Traditional physical and chemical separation technique used for large-scale concentration of omega-3 fatty is by hydrolysis processes, lipases can be employed to enhance omega-3 fatty acids in oils. They demonstrate substrate selectivity by hydrolyzing polyunsaturated fatty acid (PUFA) esters more slowly than more saturated fatty acid esters. In place of distillation and

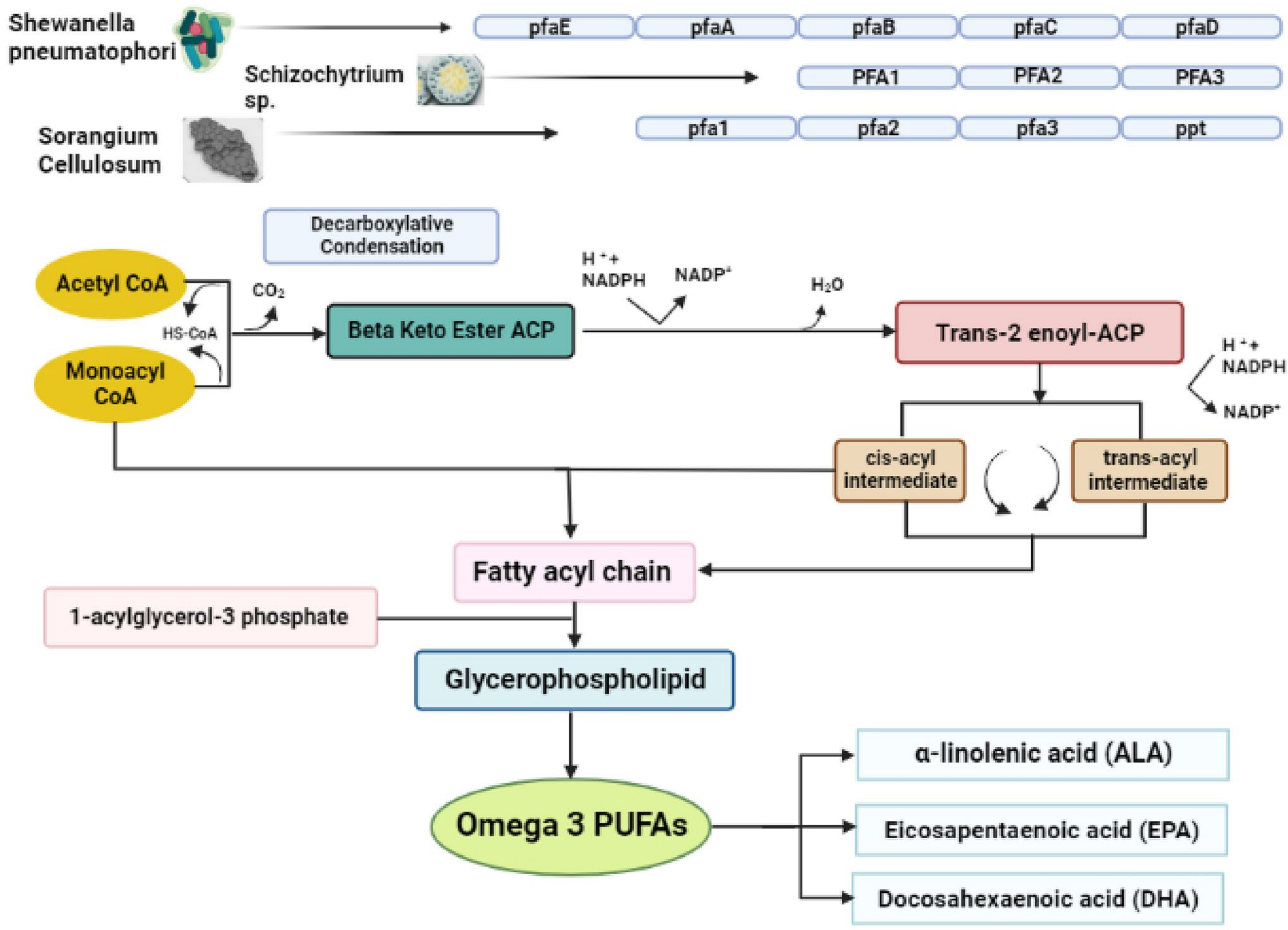

Fig. 3 Anaerobic pathway utilizing PKS-like PUFA synthases and the corresponding pfa gene clusters, eukaryotic microalgae, and bacteria employ an anaerobic pathway for the fresh production of PUFAs through de novo synthesis

chromatographic techniques, enzyme concentration provides a safe environment for reactions (Akoh 2017).

Optimization of physicochemical parameters

Oleaginous bacteria develop lipid accumulation due to nutrient imbalances in the growth medium. Nitrogen deficiency and elevated amounts of carbon sources in the medium favor the buildup of lipids in these organisms.

Carbon sources

The most significant nutrient for microbe development is carbon, which includes sugars, hexoses, pentoses, saccharides, glycosides, organic acids, and alcohols. Pyruvic acid converts the most prevalent carbon sources, like sugar molecules, *i.e.,* including (Chen et al. 2020) glucose, fructose, and glycerol, into fatty acid, which is converted into acetyl-CoA via the citric acid cycle. The molecule acetyl-CoA serves as a building block for the synthesis of lipids. Carbon substrate concentrations in culture medium range from 5 to 70 g/L in most studies, with a tolerance of up to 150 g/L (Oliver et al. 2020). The effect of carbon sources on the synthesis of DHA in *Thraustochytrium aureum* led to a maximum production of 511 mg/L in cultures containing 2.5% light-exposed starch. The effect of carbon input on DHA production by *Thrausfochytrium sp.* was investigated in shake-flask cultures maintained at 25 °C for 5 days. Equivalent biomass and lipid yields were produced using glucose, starch, and linseed oil. Most research has shown carbon substrate concentrations in the culture medium ranging from 5 to 70 g/L, with a tolerance of up to 150 g/L.

DHA synthesis was 15.24% higher in the mixed substrate conditions in the fed-batch culture with continuous air supply than glucose as the only carbon source. They evaluated the growth of *A. limacinum* with 10 g/L glucose and 83% glycerol. They found identical net biomass, growth rates, and DHA production (Chen et al. 2022b). Using the response surface approach, they optimized *Aurantiochytrium* SW1's lipid and DHA synthesis, and they found that 250 rpm agitation speed, 70 g/L fructose, and 10 g/L monosodium glutamate were the ideal conditions (Keegan et al. 2019).

Nitrogen sources

In the first phase of fermentation, nitrogen is essential for cell development, as well as the synthesis of proteins. When the fermentation medium is deprived of nitrogen, algae-like organisms produce fatty acids using a carbon source. The impact of nitrogen sources on DHA production by *T. aureum* was reported. This was accomplished using peptone, tryptone, yeast extract, malt extract, and sodium glutamate. The medium of sodium glutamate produced the highest level of DHA (269 mg/L), accompanied by 247.7 mg/L of yeast extract. The effects of nitrogen (N) sources on DHA synthesis by (ATCC 20692) *Thraustochytrium sp.,* were investigated using tryptone, malt extract, peptone, sodium glutamate and amino acids. The most effective nitrogen sources were peptone and sodium glutamate 419 mg/L and 482 mg/L, respectively (Heggeset et al. 2019).

pH

An optimum biomass content and DHA production level were found when *T. aureum* ATCC 34304 was cultivated at an initial pH of 6.0 (Bajpai et al. 1991). Subsequently, the effect of starting pH on DHA synthesis in a basal medium containing 2% glucose was also observed (Verma et al. 2020), according to which, *Thraustochytrium sp.* ATCC 20892 should have a pH of 7.0. In a controlled batch fermenter culture in glucose and maltose medium, we examined the effects of pH and aeration on the synthesis of docosahexaenoic acid by *T. aureum.* We discovered that pH 5.5 was optimal for DHA production. They discovered how the growth medium's initial pH affected the DHA production by *Thraustochytrium roseum* ATCC 28210 in basal media supplemented with 2.5% starch. They discovered that an initial pH of 6.0 was the most favorable condition. At pH 6.0, *Aurantiochytrium* species produce the most DHA (Furlan et al. 2019; Verma et al. 2020).

Temperature

Investigating two *Thraustochytrids* strains, *Schizochytrium* sp. and *Thraustochytriidae* sp., it found that the best temperature for obtaining maximum DHA production is 28 ℃ for both. They also concluded lower temperatures promote increased DHA output but lower cell biomass (Hu et al. 2021). *T. aureum* ATCC 34304 produces DHA at a temperature of 28 ℃; according to studied *Thraustochytrium sp.* ATCC 20892, it was found that 25 ℃ is the optimal temperature for DHA production. The *T. roseum* generated the highest DHA at 25 ℃ temperature. *S. limacine* (OUC88) shows the optimal temperature for cell growth, and DHA production is at 23 ℃; the ideal temperature range for the synthesis of DHA in *Aurantiochytrium sp.* SD116 is between 20 °C and 28 ℃. According to *Aurantiochytrium sp.*, strain mh0186 produces the most DHA at temperatures between 15 and 20 °C. (Bagul and Annapure 2021b).

Salinity

At all saline levels, 50% seawater content is optimal for *S. limacine* SR21 cell development; it was found that DHA synthesis at salinity level was 25% like that of seawater. The *T. aureus* strain could not be grown on a zero-salinity medium or at significantly higher saline conditions. The finest level of salinity was half the level of seawater. *C. cohn* thrives at a comparable salinity suitable for cultivation and lipid production (Yokochi et al. 1998).

Conclusion

The review emphasizes the increasing global demand for omega-3 fatty acids, particularly DHA, and the challenge of meeting this demand through traditional fish oil sources. Advances in genetic engineering and metabolic engineering techniques have enabled researchers to modify microorganisms for higher DHA yields, improved productivity, and reduced production costs. The findings of the review suggest that microbial sources hold significant promise in meeting the global demand for omega-3 fatty acids, offering an efficient and sustainable solution for enhancing human health. Microbial systems offer the potential to produce customized oils and hold promise for future biotechnological applications. In conclusion, the exploration of microbial systems as a source of omega-3 fatty acids opens up new possibilities for enhancing economic productivity and providing a sustainable solution to address the increasing demand for these essential nutrients. By leveraging the potential of microbial sources, we can contribute to improving human health while reducing the pressure on traditional fish oil sources. Further research and technological advancements in this field will likely pave the way for innovative solutions to meet the nutritional needs of a growing global population. Further research and development in this field are essential to unlock the full potential of marine microorganisms for biotechnological applications in the nutraceutical industry.

Authors' contributions NA, SM, and YS: design of the study; SR and RE: data acquisition; SA and BB: literature search of the study; SR, RE, and IP: manuscript preparation; IP and RE: critical review of the manuscript; SM and SR: finalize the revision of the manuscript. QS and SHIA: provided all the facilities.

Funding The authors received no financial support for the research authorship and publication of the article.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Akoh CC (2017) Food lipids: chemistry, nutrition, and biotechnology. CRC Press, New York

Alfio VG, Manzo C, Micillo R (2021) From fish waste to value: an overview of the sustainable recovery of omega-3 for food supplements. Molecules 26:1002

Alvarez HM, Hernández MA, Lanfranconi MP, Silva RA, Villalba MS (2021) Rhodococcus as biofactories for microbial oil production. Molecules 26:4871

Archer L et al (2019) Fatty acid profiling of new Irish microalgal isolates producing the high-value metabolites EPA and DHA. Algal Res 44:101671

Bagul VP, Annapurna US (2021a) Isolation and characterization of docosahexaenoic acid-producing novel strain *Aurantiochytrium* sp. ICTFD5: a sterol with vitamin d-cholecalciferol and cellulase and lipase-producing thraustochytrid. Bioresour Technol Rep. 14:100688

Bagul VP, Annapurna US (2021b) Isolation of fast-growing thraustochytrids and seasonal variation on the fatty acid composition of thraustochytrids from mangrove regions of Navi Mumbai. India J Environ Manag 290:112597

Bajpai P, Bajpai P, Ward O (1991) Optimization of production of docosahexaenoic acid (DHA) by *Thraustochytrium aureum* ATCC 34304. J Am Oil Chem Soc 68:509–514

Betancor M et al (2021) Oil from transgenic Camelina sativa as a source of EPA and DHA in feed for European sea bass (*Dicentrarchus labrax* L.). Aquaculture 530:735759

Branco-Vieira M et al (2020) Biotechnological potential of *Phaeodactylum tricornutum* for biorefinery processes. Fuel 268:117357

Burns-Whitmore B, Froyen E, Heskey C, Parker T, San Pablo G (2019) Alpha-linolenic and linoleic fatty acids in the vegan diet require dietary reference intake/adequate intake special consideration? Nutrients 11:2365

Chandrasekhar K et al (2022) Algae biorefinery: a promising approach to promote microalgae industry and waste utilization. J Biotechnol 345:1–16

Chaudhary A et al (2022) Genomic insights into omega-3 polyunsaturated fatty acid producing *Shewanella* sp. N2AIL from fish gut. Biology 11:632

Chen X et al (2020) Different carbon and nitrogen sources regulated docosahexaenoic acid (DHA) production of *Thraustochytriidae* sp. PKU# SW8 through a fully functional polyunsaturated fatty acid (PUFA) synthase gene (pfaB). Bioresour Technol 318:124273

Chen J, Jayachandran M, Bai W, Xu B (2022a) A critical review on the health benefits of fish consumption and its bioactive constituents. Food Chem 369:130874

Chen L, Liu X, Li C, Li H, Chen W, Li D (2022b) Transcriptome analyses reveal the DHA enhancement mechanism in *Schizochytrium limacinum* LD11 mutant. Algal Res 67:102868

Chi G et al (2022) Production of polyunsaturated fatty acids by *Schizochytrium* (*Aurantiochytrium*) spp. Biotechnol Adv 55:107897

Cui Y, Thomas-Hall SR, Chua ET, Schenk PM (2021) Development of a *Phaeodactylum tricornutum* biorefinery to sustainably produce omega-3 fatty acids and protein. J Clean Prod 300:126839

Dawood MA, Abd El-Kader MF, Farid MA, Abd-Elghany MF, Alkafafy M, Van Doan H (2021) SaccharomyceS cereviSiae enhanced the growth, immune and antioxidative responses of European seabass (DicentrarchuS labrax). Ann Anim Sci 21:1423–1433

De Abreu SL, Verneque BJF, Mota APL, Duarte CK (2021) Chia seed (*Salvia hispanica* L.) consumption and lipid profile: a systematic review and meta-analysis. Food Funct 12:8835–8849

Deeba F, Kumar KK, Gaur NA (2020) Overview of microbial production of omega-3-polyunsaturated fatty acid. Nutraceutical fatty acids from oleaginous microalgae: a human health perspective. Wiley, New York, pp 145–163

Diao J, Li X, Pei G, Liu L, Chen L (2018) Comparative metabolomic analysis of *Crypthecodinium cohnii* in response to different dissolved oxygen levels during docosahexaenoic acid fermentation. Biochem Biophys Res Commun 499:941–947

Diao J et al (2019) Rewiring metabolic network by chemical modulator-based laboratory evolution doubles lipid production in *Crypthecodinium cohnii*. Metab Eng 51:88–98

Dietzen NM et al (2022) Polyunsaturated fatty acids inhibit a pentameric ligand-gated ion channel through one of two binding sites. Elife 11:e74306

Du F et al (2021) Biotechnological production of lipid and terpenoid from thraustochytrids. Biotechnol Adv 48:107725

Eltanahy E, Shehatta B (2022) Genetic engineering of microalgae for non-fuel, high-value biochemicals of industrial significance. Microalgae for sustainable products: the green synthetic biology platform. Royal Society of Chemistry, Cambridge

Ferreira I, Rauter AP, Bandarra NM (2022) Marine sources of DHA-rich phospholipids with anti-alzheimer effect. Mar Drugs 20:662

Fu Y et al (2021) Associations among dietary omega-3 polyunsaturated fatty acids, the gut microbiota, and intestinal immunity. Mediators of Inflamm. https://doi.org/10.1155/2021/8879227

Furlan VJM, Batista I, Bandarra N, Mendes R, Cardoso C (2019) Conditions for the production of carotenoids by *Thraustochytrium* sp. ATCC 26185 and *Aurantiochytrium* sp. ATCC PRA-276. J Aquat Food Prod Technol 28:465–477

Garcia R, Stadler M, Gemperlein K, Müller R (2016) Aetherobacter *fasciculatus* gen. nov., sp. nov. and *Aetherobacter rufus* sp. nov., novel myxobacteria with promising biotechnological applications. Int J Syst Evol Microbiol 66:928–938

Gemperlein K, Zipf G, Bernauer HS, Müller R, Wenzel SC (2016) Metabolic engineering of *Pseudomonas putida* for production of docosahexaenoic acid based on a myxobacterial PUFA synthase. Metab Eng 33:98–108

Gemperlein K et al (2019) Polyunsaturated fatty acid production by Yarrowia lipolytica employing designed myxobacterial PUFA synthases. Nat Commun 10:4055

Giner-Robles L, Lázaro B, de la Cruz F, Moncalián G (2018) fabH deletion increases DHA production in *Escherichia coli* expressing Pfa genes. Microb Cell Fact 17:1–9

Guo D-S et al (2018) Development of a multi-stage continuous fermentation strategy for docosahexaenoic acid production by *Schizochytrium* sp. Biores Technol 269:32–39

Hamilton ML, Haslam RP, Napier JA, Sayanova O (2014) Metabolic engineering of *Phaeodactylum tricornutum* for the enhanced accumulation of omega-3 long chain polyunsaturated fatty acids. Metab Eng 22:3–9

Han X, Zhao Z, Wen Y, Chen Z (2020) Enhancement of docosahexaenoic acid production by overexpression of ATP-citrate lyase and acetyl-CoA carboxylase in *Schizochytrium* sp. Biotechnol Biofuels 13:1–13

Hashimoto M et al (2015) n-3 fatty acids effectively improve the reference memory-related learning ability associated with increased brain docosahexaenoic acid-derived docosanoids in aged rats. Biochim Et Biophys Acta. 1851:203–209

Heggeset TM, Ertesvåg H, Liu B, Ellingsen TE, Vadstein O, Aasen IM (2019) Lipid and DHA-production in *Aurantiochytrium* sp.–responses to nitrogen starvation and oxygen limitation revealed by analyses of production kinetics and global transcriptomes. Sci Rep 9:19470

Horrocks LA, Yeo YK (1999) Health benefits of docosahexaenoic acid (DHA). Pharmacol Res 40:211–225

Hu X, Tang X, Bi Z, Zhao Q, Ren L (2021) Adaptive evolution of microalgae *Schizochytrium* sp. under high temperature for efficient production of docosahexaenoic acid. Algal Res 54:102212

Jovanovic S, Dietrich D, Becker J, Kohlstedt M, Wittmann C (2021) Microbial production of polyunsaturated fatty acids—high-value ingredients for aquafeed, superfoods, and pharmaceuticals. Curr Opin Biotechnol 69:199–211

Karageorgou D, Rova U, Christakopoulos P, Katapodis P, Matsakas L, Patel A (2023) Benefits of supplementation with microbial omega-3 fatty acids on human health and the current market scenario for fish-free omega-3 fatty acid. Trends Food Sci Technol. https://doi.org/10.1016/j.tifs.2023.04.018

Keegan J, Currie D, Knox A, Moran C (2019) Redressing the balance: including DHA-rich *Aurantiochytrium limacinum* in broiler diets increases tissue omega-3 fatty acid content and lowers the n-6: n-3 ratio. Br Poult Sci 60:414–422

Kothari M, Mavrommati M, Elazzazy AM, Baeshen MN, Moussa TA, Aggelis G (2020) 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. https://doi.org/10.1093/femsle/fnaa028

Li Z et al (2018) Overexpression of malonyl-CoA: ACP transacylase in *Schizochytrium* sp. to improve polyunsaturated fatty acid production. J Agric Food Chem 66:5382–5391

Li J, Pora BL, Dong K, Hasjim J (2021) Health benefits of docosahexaenoic acid and its bioavailability: a review. Food Sci Nutr 9:5229–5243

Liu L et al (2018) Nitrogen feeding strategies and metabolomic analysis to alleviate high-nitrogen inhibition on docosahexaenoic acid production in *Crypthecodinium cohnii*. J Agric Food Chem 66:10640–10650

Liu Y, Ren X, Fan C, Wu W, Zhang W, Wang Y (2022) Health benefits, food applications, and sustainability of microalgae-derived N-3 PUFA. Foods 11:1883

Ma Z, Tan Y, Cui G, Feng Y, Cui Q, Song X (2015) Transcriptome and gene expression analysis of DHA producer *Aurantiochytrium* under low-temperature conditions. Sci Rep 5:14446

Malik S et al (2023) A comprehensive review on microalgae-based biorefinery as two-way source of wastewater treatment and bioresource recovery. Clean: Soil, Air, Water 51:2200044

Mun JG, Legette LL, Ikonte CJ, Mitmesser SH (2019) Choline and DHA in maternal and infant nutrition: synergistic implications in brain and eye health. Nutrients 11:1125

Nazir Y, Shuib S, Kalil MS, Song Y, Hamid AA (2018) Optimization of culture conditions for enhanced growth, lipid and docosahexaenoic acid (DHA) production of *Aurantiochytrium* SW1 by response surface methodology. Sci Rep 8:8909

Nopparatmaitree M, Nava M, Chumsangchotisakun V, Saenphoom P, Chotnipat S, Kitpipit W (2022) Effect of trimmed asparagus by-products supplementation in broiler diets on performance, nutrients digestibility, gut ecology, and functional meat production. Vet World 15:147

Ogawa T, Hirose K, Yusuf Y, Kawamoto J, Kurihara T (2020) Bioconversion from docosahexaenoic acid to eicosapentaenoic acid in the marine bacterium *Shewanella livingstonensis* Ac10. Front Microbiol 11:1104

Oliver L, Dietrich T, Marañón I, Villarán MC, Barrio RJ (2020) Producing omega-3 polyunsaturated fatty acids: a review of sustainable sources and future trends for the EPA and DHA market. Resources 9:148

Ortiz-Sanchez M, Inocencio-García P-J, Alzate-Ramírez AF, Alzate CAC (2023) Potential and restrictions of food-waste valorization through fermentation processes. Fermentation 9:274

Papanikolaou Y, Fulgoni VL III (2019) Egg consumption in US children is associated with greater daily nutrient intakes, including protein, lutein+ zeaxanthin, choline, α-linolenic acid, and docosahexaenoic acid. Nutrients 11:1137

Peng Y-F, Chen W-C, Xiao K, Xu L, Wang L, Wan X (2016) DHA production in *Escherichia coli* by expressing reconstituted essential genes of polyketide synthase pathway from marine bacteria. PLoS ONE 11:e0162861

Penno CA et al (2020) Lipidomics profiling of hidradenitis suppurativa skin lesions reveals lipoxygenase pathway dysregulation and accumulation of proinflammatory leukotriene B4. J Investig Dermatol 140(2421–2432):e2410

Perretti G, Motori A, Bravi E, Favati F, Montanari L, Fantozzi P (2007a) Supercritical carbon dioxide fractionation of fish oil fatty acid ethyl esters. J Supercrit Fluid 40:349–353

Perretti G, Motori A, Bravi E, Favati F, Montanari L, Fantozzi P (2007b) Supercritical carbon dioxide fractionation of fish oil fatty acid ethyl esters. J Supercritical Fluids 40:349–353

Petrie JR et al (2020) Development of a *Brassica napus* (Canola) crop containing fish oil-like levels of DHA in the seed oil. Front Plant Sci 11:727

Qiu X, Xie X, Meesapyodsuk D (2020) Molecular mechanisms for biosynthesis and assembly of nutritionally essential very long-chain polyunsaturated fatty acids in microorganisms. Prog Lipid Res 79:101047

Řezanka T, Kolouchová I, Mat'átková O (2020) Alternative sources of omega-3 polyunsaturated fatty acids. Stud Nat Prod Chem 67:123–159

Shahidi F (2006) Nutraceutical and specialty lipids and their co-products. Crc Press, New York

Sheikh O, Vande Hei AG, Battista A, Hammad T, Pham S, Chilton R (2019) Cardiovascular, electrophysiologic, and hematologic effects of omega-3 fatty acids beyond reducing hypertriglyceridemia: as it pertains to the recently published REDUCE-IT trial. Cardiovasc Diabetol 18:1–12

Simonato M et al (2023) DHA turnover in pregnant women using the natural abundance variation of 13C: a pilot study. Br J Nutr 129:240–246

Siriwardhana N, Kalupahana NS, Moustaid-Moussa N (2012) Health benefits of n-3 polyunsaturated fatty acids: eicosapentaenoic acid and docosahexaenoic acid. Adv Food Nutr Res 65:211–222

Sun K (2020) Functional analysis of dehydratase domains of a polyunsaturated fatty acid synthase from *Thraustochytrium* sp. by mutagenesis. University of Saskatchewan, Saskatoon

Sun X-M et al (2018) Development of a cooperative two-factor adaptive-evolution method to enhance lipid production and prevent lipid peroxidation in *Schizochytrium* sp. Biotechnol Biofuels 11:1–16

Tocher DR, Betancor MB, Sprague M, Olsen RE, Napier JA (2019) Omega-3 long-chain polyunsaturated fatty acids, EPA and DHA: bridging the gap between supply and demand. Nutrients 11:89

Turchini GM, Torstensen BE, Ng WK (2009) Fish oil replacement in finfish nutrition. Rev Aquac 1:10–57

Verma ML, Kishor K, Sharma D, Kumar S, Sharma KD (2020) Microbial production of omega-3 polyunsaturated fatty acids. Biotechnological production of bioactive compounds. Elsevier, New York, pp 293–326

Wang F, Pan X (2019) Image segmentation for the somatic milk cell based on niching particle swarm optimization Otsu. Eng Agric Environ Food 12:141–149

Wang S et al (2020) Optimizing eicosapentaenoic acid production by grafting a heterologous polyketide synthase pathway in the thraustochytrid *Aurantiochytrium*. J Agric Food Chem 68:11253–11260

Wang Z et al (2022) An auxin-like supermolecule to enhance growth and cumulative eicosapentaenoic acid production in *Phaeodactylum tricornutum*. Biores Technol 345:126564

Wang J et al (2023) Reprogramming the fatty acid metabolism of Yarrowia lipolytica to produce the customized omega-6 polyunsaturated fatty acids. Bioresour Technol 383:129231

West A, Miles E, Lillycrop K, Napier J, Calder P, Burdge G (2021) Genetically modified plants are an alternative to oily fish for providing n-3 polyunsaturated fatty acids in the human diet: a summary of the findings of a Biotechnology and Biological Sciences Research Council funded project. Nutr Bull 46:60–68

Xu Y (2022) Biochemistry and biotechnology of lipid accumulation in the microalga *Nannochloropsis oceanica*. J Agric Food Chem 70:11500–11509

Xu C, Fan J, Shanklin J (2020) Metabolic and functional connections between cytoplasmic and chloroplast triacylglycerol storage. Prog Lipid Res 80:101069

Yokochi T, Honda D, Higashihara T, Nakahara T (1998) Optimization of docosahexaenoic acid production by *Schizochytrium limacinum* SR21. Appl Microbiol Biotechnol 49:72–76

Zhang T-T, Xu J, Wang Y-M, Xue C-H (2019) Health benefits of dietary marine DHA/EPA-enriched glycerophospholipids. Prog Lipid Res 75:100997

Zhang R, Luo Y, Yu X, Dang M, Hu X, Ren L (2023) Adaptive evolution of *Schizochytrium* sp. under light and H2O2 condition to regulate its fatty acid and terpene biosynthesis. Algal Res 72:103127

Zhao B, Li Y, Li C, Yang H, Wang W (2018) Enhancement of *Schizochytrium* DHA synthesis by plasma mutagenesis aided with malonic acid and zeocin screening. Appl Microbiol Biotechnol 102:2351–2361

Zhong H, Zhang M, Chen L, Liu W, Tao Y (2023) Development of Schizochytrium sp. strain HS01 with high-DHA and low-saturated fatty acids production by multi-pronged adaptive evolution. Biotechnol Lett 45(9):1–11

Zhou Q, Wei Z (2023) Food-grade systems for delivery of DHA and EPA: Opportunities, fabrication, characterization and future perspectives. Crit Rev Food Sci Nutr 63:2348–2365