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Microbial production of polyunsaturated fatty acids — high-value ingredients for aquafeed, superfoods, and pharmaceuticals

Sofija Jovanovic, Demian Dietrich, Judith Becker, Michael Kohlstedt and Christoph Wittmann

Polyunsaturated fatty acids (PUFAs), primarily docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have received worldwide attention in recent years due to an increasing awareness of their uniqueness in improving diet and human health and their apparently inevitable shortage in global availability. Microbial cell factories are a major solution to supplying these precious molecules in sufficient amounts and providing PUFA-rich aquafeed, superfoods, and medical formulations. This review assesses the PUFA world markets and highlights recent advances in upgrading and streamlining microalgae, yeasts, fungi, and bacteria for high-level PUFA production and broadening of the PUFA spectrum.

Address
Institute of Systems Biotechnology, Universität des Saarlandes, Germany

Corresponding author:
Wittmann, Christoph (christoph.wittmann@uni-saarland.de)

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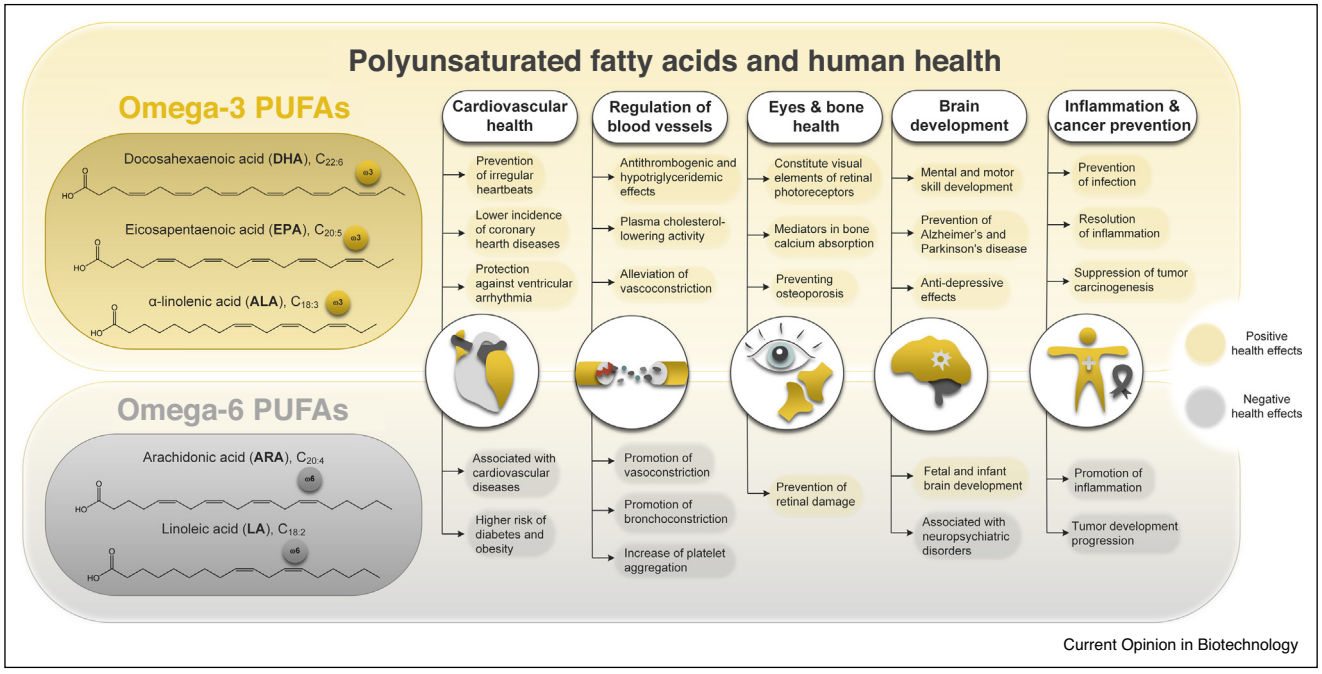
Introduction

For almost 50 years, polyunsaturated fatty acids (PUFAs) have been known as health-promoting active ingredients [1]. Pioneered by studies on rats that suggested fats are critical to health and more than interchangeable calories [2], the discovery of PUFA-related health benefits in Greenland Inuit represented the starting point of intense research efforts that changed the value of these molecules from being of only negligible interest as a drying oil constituent into health stars with a billions US$ market (Figure 1) [3]. As revealed by numerous medical studies, PUFAs are indispensable for the brain, nerves [4] and eye function [5]; prevent neurodegenerative diseases and psychiatric disorders [6]; and protect against cardiovascular diseases [7,8], infection [9,10], and cancer [11] (Figure 1). Humans cannot synthetize PUFAs *de novo*, making them essential nutrients [12]. The most beneficial (and industrially impactful) PUFAs are omega-3 derivatives, spearheaded by docosahexaenoic acid (DHA, 22:6, ω3), eicosapentaenoic acid (EPA, 20:5, ω3), and α-linolenic acid (ALA, 18:3, ω3) [13]. Omega-6 PUFAs form another significant family of dietary PUFAs. Prominent members, such as linoleic acid (LA, 18:2, ω6) and arachidonic acid (ARA, 20:4, ω6), have a complex role in metabolism [14,15]. On one hand, they appear indispensable for important cellular processes, including brain development [16] and function [17] and development of the nervous system [18], but they can also cause detrimental effects [19]. In addition, other PUFAs have become increasingly important due to their increasingly revealed importance for human health, including γ-linoleic acid (GLA, 18:3, ω6), stearidonic acid (SDA, 18:4, ω3), dihomo-γ-linoleic acid (DGLA, 20:3, ω6), eicosadienoic acid (EDA, 20:2, ω6), docosapentaenoic acid (DPA, 22:5, ω3), and very-long-chain (VLC) PUFAs, such as tetracosatetraenoic acid (TTA, 24:4, ω6), tetracosapentaenoic acid (TTA, 24:5, ω3), and tetracosahexaenoic acid (THA, 24:6, ω3) (Figure 1).

PUFA world markets: severe supply deficits have created a billion US$ market

Most vegetable oils are rich in omega-6 PUFAs. Their consumption mediates uptake to a normally sufficient level [20]. Flaxseed and chia seed oils and walnuts are rich sources of omega-3 ALA [20]. The intake of DHA and EPA, in contrast, relies on marine oily fish that feed on marine phytoplankton, such as salmon, sardines, herring, and mackerel [21], as primary producers of these PUFAs [22]. Unfortunately, the supplies of DHA and EPA from our oceans are falling because of overfishing and climate warming [23]. Wild fisheries are not keeping pace with the growing world population (Figure 2), and many are in decline, some of them exponentially so [24]. Aquaculture is meant to provide fish instead but heavily depends on the external supply itself and is a major producer and consumer of DHA and EPA at the same time [25•]. Making this situation even worse, cold-protective PUFA production in phytoplankton is diminishing due to increasing global temperature, leading to reduced contents of DHA and EPA in caught fish [26]. Present predictions for DHA estimate that its availability might decrease to levels for which more than 90% of the world's population will suffer

Figure 1



Polyunsaturated fatty acids (PUFAs) and their impacts on human health. Chemically, PUFAs are fatty acids with more than one double bond in their backbone. The distance from the first double bond from the end of the acyl chain, that is, the terminal ω-carbon atom, differs among PUFAs and allows them to be classified into omega-6, omega-3, and omega-9 PUFAs. The most important ones have at least 18 carbon atoms and belong to the group long-chain (LC) PUFAs. The most prominent omega-3 (DHA, EPA, ALA) and omega-6 PUFAs (LA, ARA) are involved in cardiovascular health [7,9], blood vessel function and control [8], eye and bone health [20], brain development [16], inflammation and cancer [10,19]. Beneficial (yellow) and detrimental (grey) effects are highlighted.

from insufficient supply [27]. Notably, we face a serious gap between supply and demand, and the deficit has been estimated at over 1 million tonnes [28].

To this end, various strategies have been proposed to enhance PUFA production, including the use of natural microbes, metabolically improved mutants derived therefrom, and strains based on conventional microbial workhorses with heterologously expressed PUFA genes and pathways (Table 1). Many microbial cell factories grow under heterotrophic conditions without light, which is beneficial towards generating high-cell-density cultures at large scales [29]. In addition to single-cell production, recent efforts (not covered here) have attempted PUFA overproduction in genetically modified crops [30] and fishes [31]. Undoubtedly, biotechnological PUFA production has received much attention from industry. PUFA-enriched oils, meals and other formulations are sold as food and feed supplements [32], whereas purified PUFAs represent high-price active ingredients of medicines and pharmaceuticals [33]. The price of single-cell oils, that is, edible oils obtainable from single-celled microorganisms, is estimated to reach 500 US$ kg^{-1} or even more, depending on the PUFA composition [34]. The world market for omega-3 PUFAs alone is estimated at US$13 billion, with a calculated annual growth rate of 8%. Among omega-6 PUFAs, the market size of ARA has been projected to reach 400 000 tonnes per year, which is worth almost US$2 billion [34].

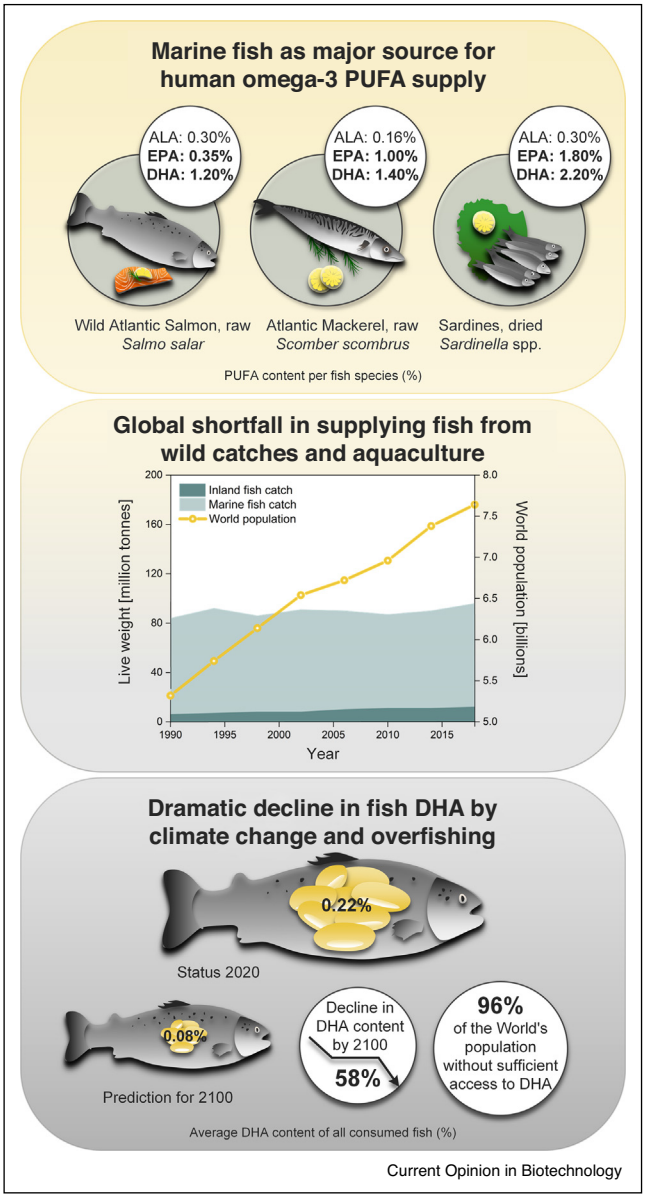
Biosynthesis of omega-3 and omega-6 PUFAs

Most organisms do not synthesize PUFAs naturally [35]. Nature has evolved two fundamental routes: aerobic (Figure 3) and anaerobic syntheses (Figure 4). The incorporation of PUFAs into the cell membrane is crucial for maintaining optimal membrane fluidity and adapting to stresses, particularly temperature, UV light, and osmotic pressure [29]. PUFAs are easily oxidized due to their high degree of unsaturation. This peroxidation is undesired because it reduces the product titre and generates reactive oxygen species (ROS) that cause severe oxidative damage to the producing cells [36].

Aerobic PUFA biosynthesis by alternating desaturation and elongation reactions

The aerobic PUFA biosynthetic pathway is (at least partially) present in animals, selected plants and eukaryotic microorganisms [37]. The last group is quite diverse and includes dimorphic fungi [38•] and various types of

Figure 2



Global view of the supply and demand of the marine PUFAs DHA and EPA. The data comprise the current PUFA contents in oily fish species, that is, salmon, mackerel, and sardines, the most important foods for DHA and EPA intake (top) [128]. The global shortage between marine PUFA supply and demand becomes obvious from the stagnation of wild fish catches [129] against the continuous growth of the human population (middle). Long-term predictions of the expected shortage of DHA supply from drastically declining DHA content in average fish consumed are shown (bottom) [27].

unicellular marine microalgae, such as photosynthetic haptophytes [39], dinoflagellates [40], and heterotrophic protists [41]. The core of the aerobic route utilizes the canonical fatty acid synthesis (FAS) machinery [42] (Figure 3) to assemble a saturated acyl intermediate that is then further extended to palmitic acid (PA), a saturated 16-carbon fatty acid, and the PUFAs ALA and LA. Alternating action of desaturases and elongases modifies and extends the backbones of ALA and LA and yields the omega-3 derivatives EPA and DHA (for ALA) and the omega-6 derivative ARA (for LA) [43]. Alternative routes exist for the initial two steps of these conversions: the Δ6 pathway [26] and the alternative Δ8 pathway [44] (Figure 3). Mammalian cells cannot incorporate double bonds into the ω3 and ω6 positions on the fatty acid backbone, so they cannot create LA and ALA. From that point in the pathway onwards, they possess all the enzymes required to form longer and more-unsaturated PUFAs, including DHA, EPA, and ARA. This capacity, however, is extremely low in humans and regarded as insufficient from a medical perspective [35]. Even large amounts of dietary ALA do not impact plasma DHA, an effect paralleled by negligible effects of dietary LA on plasma ARA [45].

Anaerobic PUFA biosynthesis via enzymes analogous to polyketide synthases

While marine bacteria synthetizing EPA and DHA have been known since the 1980s, it was long assumed that they use the common aerobic pathway. Efforts in cloning EPA biosynthetic genes [46] and the subsequent discovery of a novel anaerobic route for bacterial PUFA biosynthesis [47] were therefore milestones in PUFA research. The anaerobic route is fundamentally different from the aerobic pathway (Figure 4). It is not integrated with FAS but uses a PUFA synthase, a polyketide synthase (PKS)-like enzyme complex, to synthesize PUFAs *de novo*. The route involves iterative cycles of condensation, dehydration, and keto-reduction [48] and also length control by a small regulator called the chain length factor [49]. Anaerobic PUFA biosynthesis has been discovered in bacteria [50,51] and unicellular microalgae [52]. It requires almost 50% less NAD(P)H than the aerobic route, which is attractive for PUFA overproduction [53••]. The different domains of PUFA synthase are encoded by *pfa* gene clusters. These clusters comprise between 3 and 5 genes, for example, *pfa123* in *Schizochytrium* spp. and in myxobacteria [54], and *pfaABCDE* in *Shewanella* spp. [49].

Marine microalgae — natural specialists for high-level production

Marine microalgae are noteworthy for their capacity to store large amounts of PUFAs in neutral triacylglycerides (TAGs) and cell membrane lipids. Prominent heterotrophic members that accumulate high levels of DHA are unicellular thraustochytrids (*Schizochytrium*, *Aurantiochytrium*, and *Thraustochytrium*) [55] and the dinoflagellate *Crypthecodinium cohnii* [56]. Although regarded as microalgae, these microbes have lost photosynthesis and live as saprophytic decomposers in the marine realm [57]. Photosynthetic microalgae, such as *Phaeodactylum tricornutum* [58], *Nannochloropsis oceanica* [59,60], and *Dunaliella salina* [61], primarily synthesize EPA [62]. Other species,

Table 1

Microbial production of omega-3 and omega-6 PUFAs in selected hosts. The cells used for production are classified into natural isolates ( ), mutagenized strains ( ), and strains metabolically engineered in aerobic PUFA biosynthesis ( ), anaerobic PUFA biosynthesis ( ), and pathways supporting precursor supply, lipid metabolism, and stress resistance ( ). For advanced strains, the best performances for each desired PUFA is highlighted in bold. The circles with two colours refer to strains engineered at two different levels

Product	Cell	Titre [g L^{-1}]	Content [% TFAs]	Content [% DCW]	Ref.
Schizochytrium sp.					
DHA		**47.4**	42.9	26.0[a]	[74]
DHA		39.2	42.9	**30.5**	[69]
DHA		38.3	**54.5**	/[b]	[65]
DHA		38.1	53.3	30.1[a]	[66]
DHA		14.0	50.9	30.0[a]	[70]
DHA		3.5	37.3[a]	26.7	[71•]
EPA		**2.3**[a]	**3.9**	**2.01**[a]	[73]
Aurantiochytrium sp.					
DHA		**12.5**[a]	23.9[a]	13.2[a]	[75]
DHA		10.0[a]	**52.5**	**28.9**[a]	[76]
DHA		4.8	52.0	25[a]	[77]
Crypthecodinium cohnii					
DHA		**13.1**[a]	52.4	26.2[a]	[80]
DHA		9.6	24.2[a]	13.5[a]	[81]
DHA		2.7[a]	**54.0**[a]	**33.8**[a]	[79]
Isochrysis galbana					
DHA		/	9.2	2.1	[39]
DHA		0.02[a]	2.7	/	[125]
Phaeodactylum tricornutum					
EPA		/	**36.5**	0.8	[58]
EPA		/	32.0	/	[85]
EPA		/	15.2	/	[86]
ARA		/	**7.5**	/	[86]
EPA[c]		/	26.2	/	[87••]
Dunaliella salina					
EPA		0.6	**46.2**[a]	21.3[a]	[61]
Nannochloropsis oceanica					
EPA		0.2[a]	/	4.1	[60]
EPA		0.02[a]	4.1	/	[59]
Chlorella vulgaris					
LA		0.03	25.3	/	[63]
ALA		0.01	10.8	/	[63]
Mortierella alpina					
ARA		**13,500**	**61.1**	**32.7**[a]	[102]
EPA		**1,900**	31.5	12.7	[104]
Mucor circinelloides					
GLA		**180**	43.0	/	[106]
GLA		**300**[a]	21.6	3.3[a]	[38•]
DGLA		**75**	5.2	0.9[a]	[38•]
Yarrowia lipolytica					
EPA		/	**56.0**	**22.2**	[95]
DHA		**350**	**16.8**	**1.7**[a]	[53••]
ALA		**1,400**	**17.0**	**7.0**	[99]
ARA		120	0.8	1.2	[98]
GLA		**1,780**	9.0	/	[97]
DGLA		**160**	0.8	/	[97]
Saccharomyces cerevisiae					
EDA		/	5.6	/	[100]
Marine bacteria spp.					
DHA		120	10.1	/	[112]
EPA		82	25.5	3.0[a]	[113]
EPA		/	4.3	/	[114]
Cyanobacteria spp.					
ALA		40[a]	53.1	/	[107]
ALA		/	22.6	/	[108]
SDA		/	26.6	/	[109]
Myxobacteria spp.					
DHA		/	11.9	/	[54]
EPA		/	10.3	/	[54]
LA		/	10.0	/	[50]

Table 1 (*Continued*)

Product	Cell	Titre [g L^{-1}]	Content [% TFAs]	Content [% DCW]	Ref.
Pseudomonas putida					
DHA		3	/	/	[120]
Escherichia coli					
DHA		20	31.7	/	[126]
DHA		2	7.0	/	[127]

[a] Estimated from reference.
[b] Not available from reference.
[c] This strain co-produced a fungal phytase.

including the green algae *Chlorella vulgaris*, mainly store LA and ALA [63].

Schizochytrium and *Aurantiochytrium* — DHA-rich microalgae

Schizochytrium spp. are the best-performing cell factories for DHA production (Table 1). Proprietary strains have been used for commercial production of DHA-rich oils and meals for more than 25 years [64]. The microbes possess aerobic and anaerobic PUFA routes but lack Δ12-desaturase, so DHA is presumably formed anaerobically. Recently, the oxygen sensitivity of DHA production was studied. Supplementation with ascorbic acid as an antioxidant decreased the level of intracellular ROS and increased the DHA titre to 38.3 g L^{-1} [65]. Adaptive laboratory evolution (ALE) resulted in the mutant ALE-TF30, which exhibited reduced lipid peroxidation and accumulated DHA with a titre of 38.1 g L^{-1}, 57% more than that in the parent strain [66]. In addition, overexpression of superoxide dismutase (*SOD1*) reduced the ROS level [67] (Figure 5a). On the bioprocess side, recent studies improved media [68] and demonstrated DHA production at the 7000 L scale [69]. Atmospheric and room temperature plasma mutagenesis (combined with resistance selection against malonic acid and zeocin) generated a mutant that produced 14 g L^{-1} DHA [70]. Heterologous expression of malic enzymes and C16/18 fatty acid elongase raised the intracellular levels of NADPH and acetyl-CoA, which are required for PUFA biosynthesis [71•] (Figure 5a). On the other hand, the latest developments have focused on increasing the contents of EPA and DHA to meet the intake recommendations for these fatty acids. An interesting approach restructured the PUFA PKS [72]. Replacement of the natural acyltransferase (AT) domain by a homologue from the EPA-producing bacterium *Shewanella* sp. beneficially changed the lipid composition (Figure 5a), which was then used to produce DHA and EPA at levels up to 28.8 and 2.3 g L^{-1}, respectively [73]. Furthermore, overexpression of malonyl-CoA ACP transacylase (MAT) shifted carbon flux towards PUFA synthesis [74]. Recent studies with *Aurantiochytrium* (formerly assigned to *Schizochytrium*) have mainly addressed fundamental aspects regarding the effects of nutrient starvation [75], low temperature [76], and carbon sources on DHA production [77]. Moreover, attempts have revealed the biosynthesis of VLC-PUFAs and have identified phosphatidylcholine as an intermediate before incorporation into TAG [78].

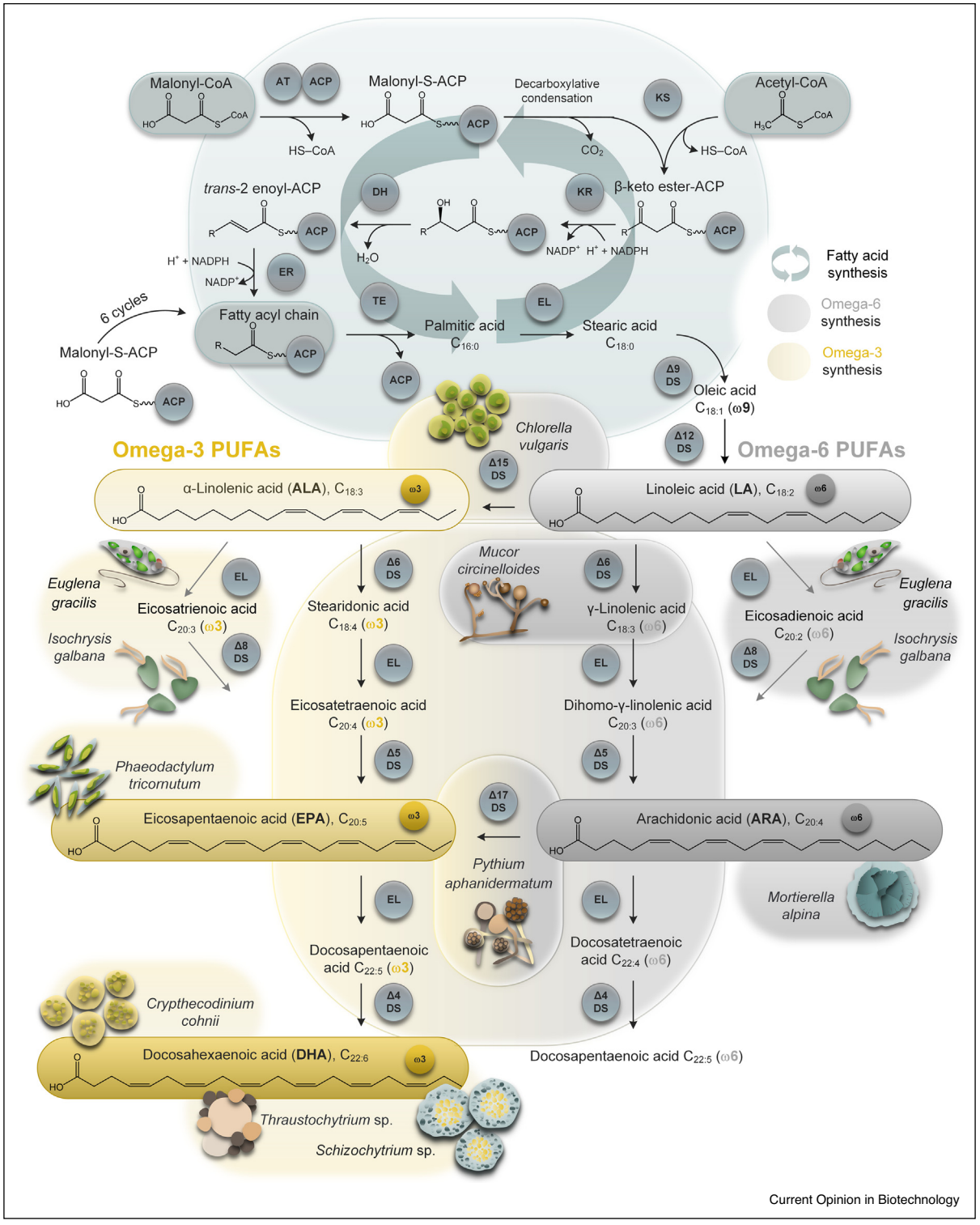
Crypthecodinium cohnii as a newly emerging DHA source

C. cohnii almost exclusively contains DHA in its lipids. An evolved strain was recently derived by chemical modulator-based ALE [79]. DHA production was enhanced to 7.8 g L^{-1} by an increase in oxygen availability [80]. Likewise, optimized nitrogen feeding elevated the DHA titre [81]. A first study recently aimed to infer metabolic fluxes in *C. cohnii* using the METAFoR approach [82•].

Phaeodactylum tricornutum — a promising diatom cell factory for EPA

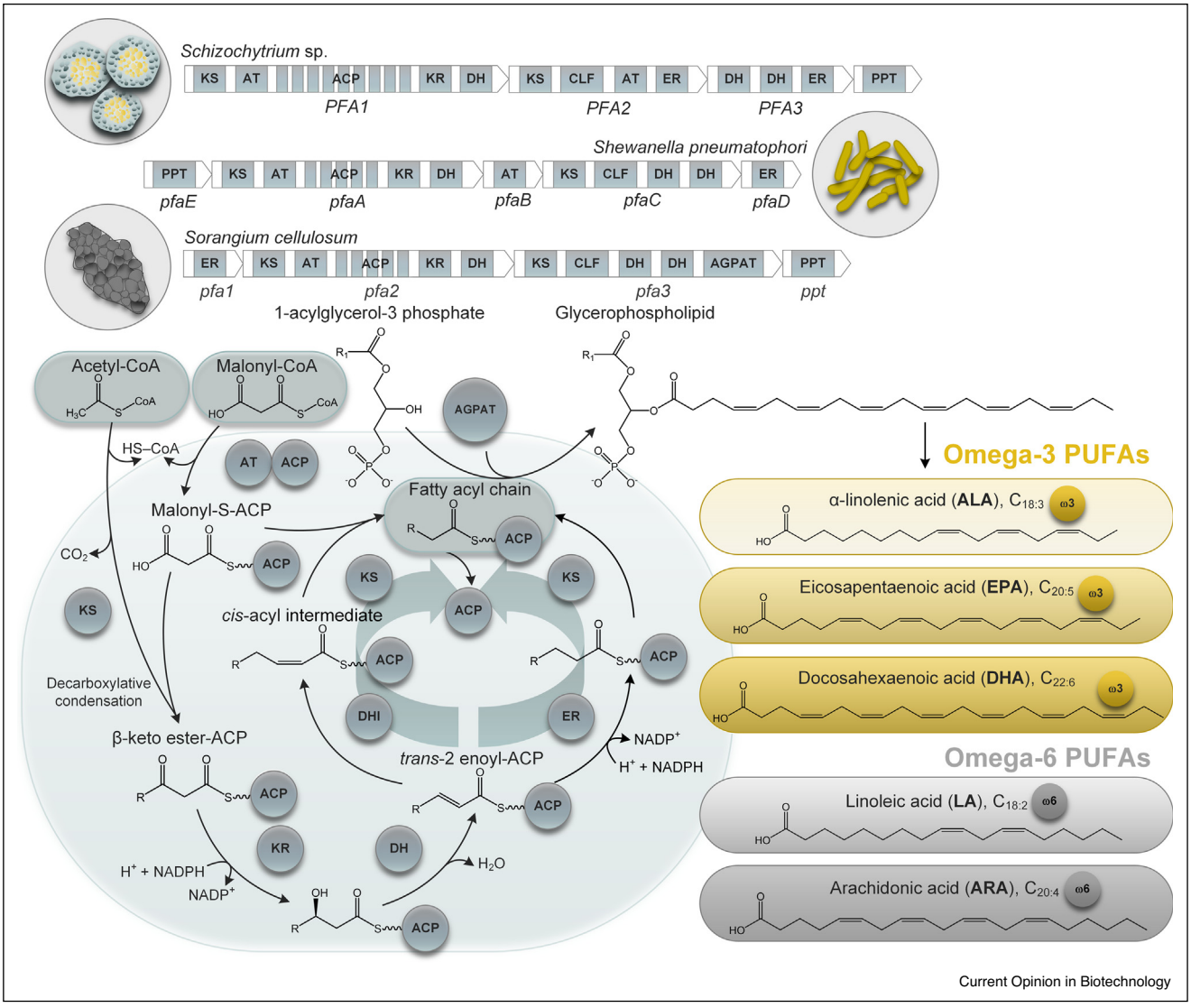
The photosynthetic diatom *P. tricornutum* naturally accumulates up to 36% EPA in its total fatty acids (TFAs) [83] (Figure 5b). A breakthrough towards using cultures without light was a trophic conversion to growth on glucose in the dark that was accomplished by introducing a glucose transporter [84]. A combined introduction of Δ5 elongase and a glucose transporter resulted in a mutant strain that accumulated 36.5% EPA and 23.6% DHA, both represented as fractions of the TFAs [58]. As further shown, overexpression of endogenous Δ6-desaturase increased the selectivity for EPA [85]. Overexpression of the native gene *AGPAT*, encoding 1-acyl-sn-glycerol-3-phosphate acyltransferase, affected primary metabolism. The total lipid content was boosted 1.8-fold, with a significant increase in TAGs components: 15.2% EPA, 7.5% ARA, and 1.2% DHA were achieved [86]. Interestingly, the mutant exhibited an increased abundance of lipid-rich droplets in the cytosol and plastids. More recently, *P. tricornutum* was upgraded to co-produce PUFAs and two widely used types of phytases in the fish food industry [87••] (Figure 5b). The final strain accumulated EPA (26.2% of TFAs), DHA (11.1%), and DPA (1.5%), together with 40 000 phytase activity units per gram of soluble protein. This development is promising for fortifying the nutritional value of vegetable feeds rich in phytate and low in DHA and EPA for use in aquaculture.

Figure 3



Aerobic pathway for the synthesis of PUFAs integrated into the fatty acid synthesis (FAS) machinery in animals, selected plants, and eukaryotic microbes by alternating elongation (chain length extension by two carbon units) and desaturation reactions (oxygen-dependent regioselective introduction of unsaturated bonds). The FAS operates either as one large multifunctional polypeptide (type I FAS in animals, yeast, and fungi) or as a complex of different mono-functional enzymes (type II FAS in prokaryotes and plants). Prominent natural PUFA-accumulating strains include LA- and ALA-producing *Chlorella vulgaris* [63], EPA-producing *Phaeodactylum tricornutum* [58,130], DHA-*producing Schizochytrium* sp. [37], *Thraustochytrium* sp. [55], *Crypthecodinium cohnii* [80], GLA-producing *Mucor circinelloides* [106] and ARA-producing *Mortierella alpina* [131]. In

Figure 4



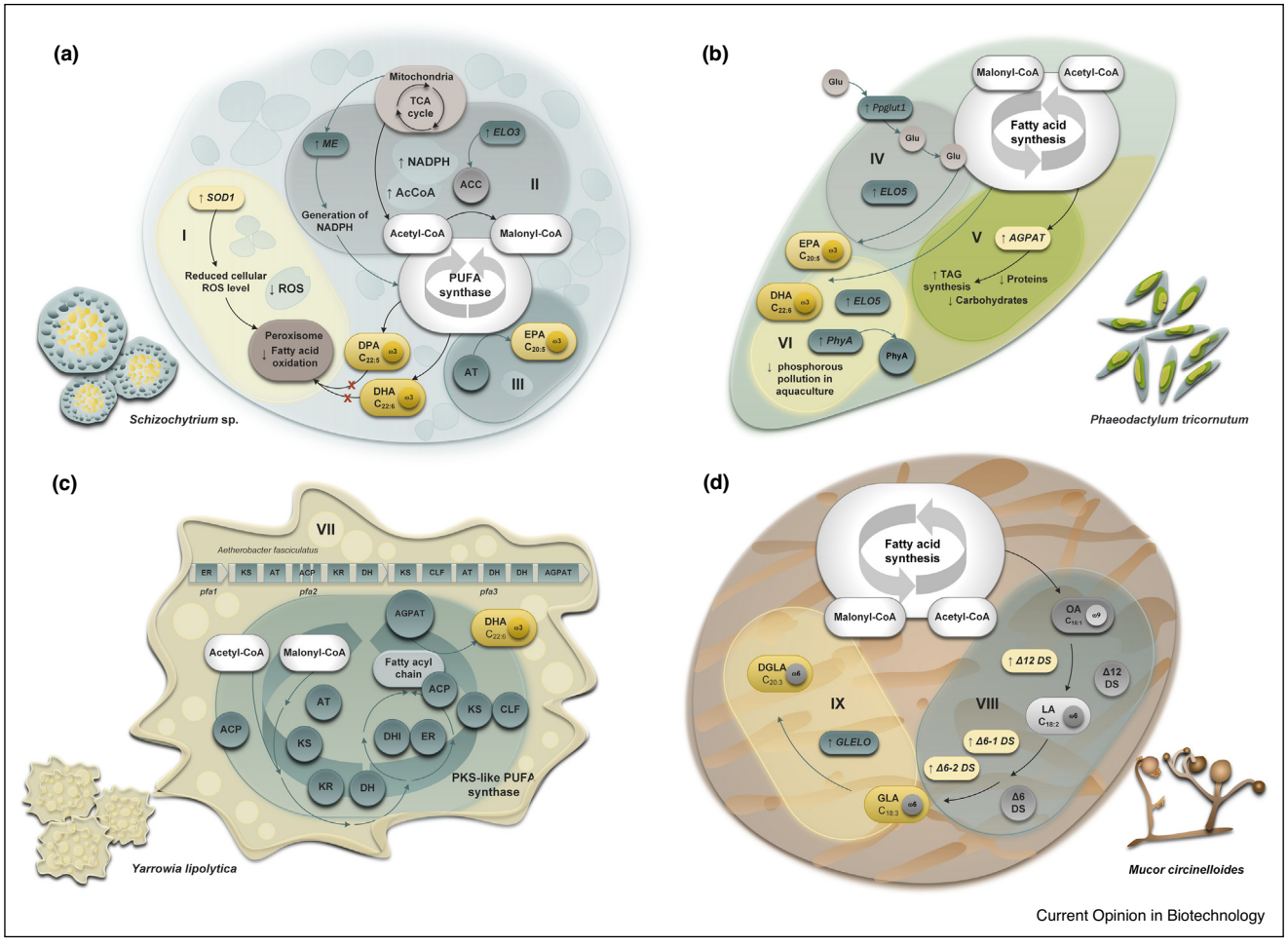
Anaerobic pathway for *de novo* synthesis of PUFAs in eukaryotic microalgae and bacteria using PKS-like PUFA synthases and corresponding *pfa* gene clusters. Abbreviations: ER: enoylreductase; KS: ketosynthase; AT: acyltransferase; DH: dehydratase; ACP: acyl carrier protein; KR: ketoreductase; DHI: dehydratase/isomerase; CLF: chain length factor; AGPAT: 1-acylglycerol-3-phosphate acyltransferase; PPT: 4′-phosphopantetheinyl transferase.

Recombinant yeast and fungi as tailor-made PUFA producers

Dimorphic yeasts and fungi such as *Yarrowia lipolytica*, *Mortierella alpina*, and *Mucor circinelloides* benefit from naturally efficient lipid metabolism and built-in protection against the toxic side effects of PUFA biosynthesis. It is thus not surprising that these microbes are among the world's top PUFA producers. Even better, the catalogue of producers is continuously expanding. For example, *Ashbya gossypii* [88•], *Rhodosporidium toruloides* [89•], and other oleaginous yeasts have recently revealed promising potential [90].

addition, selected microbes produce unique enzymes that have been used to upgrade PUFA production in heterologous hosts, such as Δ8-desaturase containing *Euglena gracilis* [94], *Isochrysis galbana* [98], and Δ17-desaturase containing *Pythium aphanidermatum* [103]. Abbreviations: ER: enoylreductase; KS: ketosynthase; AT: acyltransferase; ACP: acyl carrier protein; KR: ketoreductase; DH: dehydratase; TE: thioesterase; DS: desaturase; EL: elongase.

Figure 5



Recent advances in metabolic engineering of microbial PUFA production. The created cell factories include strains of *Schizochytrium* sp. **(a)** with (i) enhanced redox power and precursor supply [71•], (ii) reduced ROS level [67], and (iii) enhanced production of EPA and the major product DHA [72]; *P. tricornutum* **(b)**, (iv) producing EPA and DHA from glucose in the dark [58], (v) exhibiting an increased lipid content with additional lipid droplets in the cytosol and the plastids [86], and (vi) co-producing PUFAs together with a fish food phytase [87••]; *Y. lipolytica* **(c)**, (vii) expressing an anaerobic NADPH-efficient PUFA *de novo* synthase for high-level DHA production [53••]; and *M. circinelloides* **(d)**, (viii) overexpressing native desaturates [106] and (ix) a heterologous elongase towards enhanced GLA and DGLA formation [38•].

Yarrowia lipolytica — the star among heterologous PUFA producers

Y. lipolytica, well known for its ability to grow in hydrophobic environments [91], accumulates massive amounts of lipids that are greatly enriched in unsaturated fatty acids [92] (Figure 3). This potential has been impressively utilized in recent years [93]. A milestone was the development of strains that produce EPA at the commercial level [94]. For this purpose, various combinations of heterologous enzymes belonging to the alternative aerobic Δ8 pathway from different native producers were implemented and tested. The development resulted in EPA accumulation accounting for up to 56.6% of TFAs and 15.0% of cell dry weight, with saturated fatty acids remaining below 5%. Inactivation of peroxisome biogenesis via deletion of the *PEX10* gene and improvement of the fermentation process finally boosted EPA production to 25.0% by weight [95].

Further efforts revealed a tremendous tunability of the yeast to produce other PUFAs. For example, the expression of Δ6-desaturase enhanced the GLA level to 6.2% of TFAs [96]. Recombinant *Y. lipolytica* expressing codon-optimized Δ6-desaturase, Δ5-desaturase, and Δ6-elongase produced ARA and its metabolic intermediates DGLA and GLA [97]. Terminal pathway engineering increased the ARA level [98]. Moreover, the introduction of a bifunctional Δ12/Δ15-desaturase in combination with low-temperature fermentation resulted in the accumulation of almost 1.4 g L^{-1} ALA [99]. A recent study demonstrated that engineered *Y. lipolytica* can synthetize DHA *de novo* utilizing a PKS-like PUFA synthase (Figure 5c). Insertion of codon-optimized

and refactored/hybrid myxobacterial gene clusters provided mutants with DHA accounting for up to 16.8% of TFAs. A specific *pfa* cluster containing the *pfa1*, *pfa2*, *pfa3* and *ppt* domains provided up to 350 mg L^{-1} DHA, which was supported by an optimized fermentation process [53••]. The advantage of using PUFA synthases for DHA synthesis as opposed to the aerobic pathway lies in an almost 50% lower demand for NAD(P)H.

Saccharomyces cerevisiae is still in its infancy regarding PUFA accumulation

S. cerevisiae produces saturated and monounsaturated fatty acids but no PUFAs. Recent attempts have demonstrated the production of EDA, LA and ALA in recombinant *S. cerevisiae* [100], but the achieved performance is far below that of other microbes (Table 1). *S. cerevisiae* is intoxicated when expressing PUFAs and exhibits damaged proteins, lipid peroxides and even caspase-mediated cell death [101]. It seems that substantial engineering is needed to streamline yeast for high-level PUFA production.

Mortierella alpina — the world's best ARA producer

M. alpina accumulates high levels of ARA, and its ARA-rich oil obtained GRAS status from the US FDA in 2001 [102]. It is not surprising that strains of *M. alpina* are still intensively studied, aiming at either higher ARA titres or a broadened PUFA spectrum (Table 1). Recent attempts have provided a multi-stage fermentation bioprocess that produces 13.5 g L^{-1} ARA [102]. Increased selectivity for EPA was achieved by partially converting ARA into its omega-3 counterpart. The introduction of a specific Δ17-desaturase resulted in an accelerated conversion of ARA into EPA and the accumulation of the latter to 1.7 g L^{-1} [103]. Similarly, heterologous expression of *PPD17*, another desaturase with high Δ17 selectivity, increased the EPA titre to 1.9 g L^{-1}, whereby EPA represented 31.5% of TFAs [104]. An alternative strategy used a mixed culture concept. Specifically, the ARA-accumulating species *Mortierella elongata* was co-cultured with the EPA-producing microalga *Nannochloropsis oceanica*, which resulted in bio-flocculation of the two microbes to enable low-cost separation [105].

High-level GLA production in *Mucor circinelloides*

Recent optimization aimed to convert the upstream pathway intermediate oleic acid (OA, present in significant amounts) into GLA (Figure 5d). For this purpose, native desaturases catalysing the desired conversion were overexpressed. The obtained mutant exhibited an elevated GLA titre of 180 mg L^{-1} and a GLA content corresponding to 43% of TFAs [106]. Regarding downstream steps in the PUFA pathway, a recent study created the first DGLA-producing mutant. Heterologous expression of Δ6 elongase, responsible for chain elongation of GLA into DGLA, enabled the production of up to 75 mg L^{-1} DGLA along with 300 mg L^{-1} GLA [38•].

Bacterial systems cannot compete in PUFA production but have emerged as valuable pathway donors

Selected bacteria perform anaerobic PUFA biosynthesis (Figure 4). Likewise, different cyanobacteria form a spectrum of PUFAs [107–109]. Recent progress revolves around the discovery of novel species and first attempts to improve PUFA production, which are admittedly still at a proof-of-concept level. Strains of *Colwellia*, heterotrophs found in cold environments [110], synthesize DHA that accounts for up to 17% of TFAs [111]. The addition of cerulenin (a FAS inhibitor) increased DHA levels in *Colwellia psychrerythraea* 34H [112]. *Shewanella* sp., another deep-sea bacterium, accumulated sufficient EPA to account for up to 36% of TFAs [113]. Supplementation with cerulenin enhanced the production of EPA by *Shewanella electrodiphila* MAR441, and chemical mutagenesis increased production at low temperature [113]. Genetically highly similar strains of *Vibrio* spp. differed in their capability to produce EPA, indicating distribution of the gene cluster by horizontal gene transfer [114]. Earlier reports revealed that other marine psychrophiles naturally accumulate high levels of PUFAs, but the lack of studies over the past few years indicates that these strains have not undergone further improvement [115]. Regarding terrestrial myxobacteria, *Aetherobacter* spp. produced all major PUFAs [54], whereas *Sorangium cellulosum* produced only LA [116], and *Phaselicystis flava* was enriched in ARA [117] (Table 1). In addition, several studies proved the feasibility of producing PUFAs in heterologous bacteria such as *Escherichia coli* [118], lactic acid bacteria [119], and *Pseudomonas putida* [120], but the production did not surpass the milligram scale.

Conclusions

PUFAs have entered the forefront of industrial biotechnology. The enormous impact of PUFAs on human health and the anticipated near-future supply/demand gap have created a market with lucrative opportunities. At this stage, PUFA-producing microbes have great potential for industrial application. Considering recent trends, we can expect tailor-made strains that surpass industrially used isolates and might even approach theoretical limits. Towards industrial implementation, bioprocess operation must be further optimized to reduce cost and increase sustainability, for example, by using waste streams and non-food renewables [121,122,123•,124]. Finally, we should continue searching for novel PUFA-rich microbes from nature to contribute to this field.

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

Sofija Jovanovic: Writing - original draft, Visualization, Writing - review & editing. **Demian Dietrich:** Writing -

review & editing. **Judith Becker:** Writing - review & editing. **Michael Kohlstedt:** Writing - review & editing. **Christoph Wittmann:** Conceptualization, Writing - original draft, Visualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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