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MINI-REVIEW

LC-PUFA from photosynthetic microalgae: occurrence, biosynthesis, and prospects in biotechnology

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Abstract Microalgae offer potential for numerous commercial applications, among them the production of long-chain polyunsaturated fatty acids (LC-PUFAs). These valuable fatty acids are important for a variety of nutraceutical and pharmaceutical purposes, and the market for these products is continually growing. An appropriate ratio of LC-PUFA of the ω-3 and ω-6 groups is vital for "healthy" nutrition, and adequate dietary intake has strong health benefits in humans. Microalgae of diverse classes are primary natural producers of LC-PUFA. This mini-review presents an introductory overview of LC-PUFA-related health benefits in humans, describes LC-PUFA occurrence in diverse microalgal classes, depicts the major pathways of their biosynthesis in microalgae, and discusses the prospects for microalgal LC-PUFA production.

Keywords Arachidonic acid · Desaturase · Docosahexaenoic acid · Eicosapentaenoic acid · LC-PUFA · Microalgae

Introduction

Fatty acids are long aliphatic carbon chains that vary in length, degree of unsaturation, and structure. Long-chain polyunsaturated fatty acids (LC-PUFAs) of 20 and 22 carbons in length (C_{20}–C_{22}) with two to six methylene-interrupted double bonds perform vital functions in the human organism: as such, they are of high physiological and therapeutic significance for human well being. The two main families of PUFA are distinguished by the distance of the last double bond from the methyl end of the acyl chain: ω-3 (or alternatively *n*-3) designates a PUFA whose last double bond is located three carbons from the ω end of the carbon chain. The essential plant-derived C_{18} PUFA (18 carbon fatty acids with two or more double bonds), namely, linoleic acid (LA, 18:2ω-6) and α-linolenic acid (ALA, 18:3ω-3), are precursors for the LC-PUFA of the ω-6 group arachidonic acid (ARA, 20:4ω-6) and of the ω-3 group eicosapentaenoic acid (EPA, 20:5ω-3) and docosahexaenoic acid (DHA, 22:6ω-3)—which are synthesized via alternating desaturation and elongation steps (Abbadi et al. 2004; Cohen et al. 1992; Meyer et al. 2004; Wallis et al. 2002 and references therein). Biosynthesis of the highly unsaturated DHA from EPA in mammals involves the formation of polyunsaturated C_{24} intermediates by sequential elongation and desaturation, followed by a β-oxidation step of 24:6ω-3 to 22:6ω-3 in the peroxisomes (Sprecher 2000). The capability of human enzymes to desaturate and elongate essential LA and ALA to EPA and, especially, to DHA is low and is not sufficient to provide an adequate supply of LC-PUFA for maintenance of mental and cardiovascular health. In addition, this capacity deteriorates with age and under some disease conditions (Bairati et al. 1992; Connor et al. 1993; Eritsland et al. 1996; Horrobin and Huang 1987; Kalmijn et al. 2004; Le et al. 2009).

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Therefore, recent opinion suggests that ARA and DHA should also be considered essential. Consequently, an adequate dietary intake exerts strong health benefits, and an appropriate ratio of LC-PUFA of the ω-3 and ω-6 groups is vital for "healthy" nutrition and brain function (Okuyama et al. 1996; Salem et al. 2001; Simopoulos 2002; Sontrop and Campbell 2006). In adults, unbalanced intake of LC-PUFA of the two groups—mainly the insufficient uptake of ω-3 LC-PUFA that is common in Western diets—results in a higher than recommended ω-6/ω-3 ratio, which coincides with an increase in the occurrence of cardiovascular disease and increased risk of cardiac death and mental illness (Aarsetoey et al. 2011; Bousquet et al. 2008; Das 2003; Simopoulos 2008; Thies et al. 2003). ARA and EPA are precursors to distinct groups of biologically active eicosanoids: prostaglandins, leukotrienes, tromboxanes, and lipoxines, which differentially affect various physiological and biochemical processes, such as inflammation, signaling, and vasomodulation (Gill and Valivety 1997; Funk 2001; Le et al. 2009; Simopoulos 2002). Prostaglandins derived from EPA via the action of cyclogenase are considered anti-inflammatory, while those produced from ARA possess pro-inflammatory properties. The biosynthetic precursor of ARA, dihomo-γ-linolenic acid (DGLA, 20:3ω-6), is a precursor of a group 1 prostaglandin (PGE_1), which has anti-inflammatory activity, and thus has potential for use in treating conditions with an inflammatory component, such as atopic eczema, psoriasis, asthma, and arthritis (Fan and Chapkin 1998).

ARA and DHA are the major constituents of brain membrane phospholipids, and their adequate supply improves visual acuity and infant cognitive development (Agostoni et al. 1998; Boswell et al. 1996; Carlson et al. 1993; Koletzko and Braun 1991; Makrides et al. 1995). The need for a dietary supply of DHA and ARA in infants, both preterm and at term, is now being met by the incorporation of both ARA and DHA into baby formulae; this recommended approach is based on recent medical studies and is becoming a common practice in many countries.

At present, the major resource of ω-3 LC-PUFA for human nutrition is marine fish oils. However, their reserves are dwindling, and supplies via wild fish fisheries are limited. Moreover, ω-3 LC-PUFA content of oil from wild-caught fish varies with species, location, water depth and temperature, seasonal climatic conditions, and availability and type of primary food chain. Because of pollution with toxic contaminants, fish oils are often not of sufficient quality to benefit human health (Kris-Etherton et al. 2002). The consequences of the recent nuclear disaster in Japan raise concerns over the world and strengthen the need to seek for an alternative source for fish oil. Furthermore, aquaculture farming also requires fish oils for the enrichment of live feed and fish feed. Worldwide attempts are being made to improve the current sources of ω-3 LC-PUFA and to provide a sustainable supply of dietary EPA and DHA. Intensive efforts are thus underway to develop alternative feedstocks by means of metabolic engineering, in particular to reconstitute the LC-PUFA biosynthetic pathway in oilseed plants, which are intrinsically unable to synthesize LC-PUFA (reviewed in Venegas-Calerón et al. 2010), and in oleaginous yeast species (Zhu et al. 2010), employing genes isolated from different LC-PUFA-producing organisms, including microalgae. Considerable progress has been made toward this goal, and recent research has provided proof of concept for the production of ω-3 LC-PUFA in transgenic plants (Napier 2007; Qi et al. 2004; Petrie et al. 2010b; Wu et al. 2005) and yeast (Zhu et al. 2010).

Several commercial single-cell sources for LC-PUFA have been developed in the last two decades. ARA is currently produced on a large scale by cultivation of the oleaginous filamentous fungus *Mortierella alpina* (Zygomycetes) to meet the increasing demands of the baby formula industry (Kyle 1997; Sakuradani et al. 2009 and references therein). Large-scale production of DHA-rich oil by Martek Biosciences and DSM is being achieved via heterotrophic cultivation of the marine dinoflagellate microalga *Cryptocodinium cohnii* (Kyle 1996, 2001). DHA-rich oil of *C. cohnii* and ARA-rich oil of *M. alpina* are included in baby formulae in many countries. Aspects of heterotrophic LC-PUFA production by microalgae have been thoroughly reviewed (Perez-Garcia et al. 2011; Sijtsma and De Swaaf 2004; Vazhappilly and Chen 1998; Wen and Chen 2003), and the present mini-review will focus mainly on photoautotrophic eukaryotic LC-PUFA-producing microalgae.

Occurrence of LC-PUFA in microalgae

Microalgae (single-celled eukaryotic organisms) are the primary natural producers of LC-PUFA. These organisms offer a promising vegetative and non-polluted resource for biotechnology and bioengineering of LC-PUFA production as an alternative to fish oil. Diverse photosynthetic and heterotrophic, mainly marine planktonic species belonging to different classes produce LC-PUFA of the ω-3 family—EPA and DHA (Table 1). Microalgal LC-PUFAs are transferred through food webs, enriching aquatic organisms with these important membrane components. This is especially important in the marine food web because of the marine fish's limited capacity to synthesize LC-PUFA de novo from the essential LA and ALA. EPA and DHA, as well as a certain level of ARA, are required for optimal nutrition and stress tolerance of marine fish, especially at the larval and juvenile stages (Bell and Sargent 2003, and references therein; Harel et al. 2002).

Table 1 Examples of LC-PUFA occurrence in various microalgae classes

Class	Genus species	Major LC-PUFA	References
Bacillariophyta (diatoms)			
Bacillariophyceae	*Phaeodactylum tricornutum*	EPA	Arao et al. 1994
			Molina-Grima et al. 1999
	Nitzschia laevis		Tan and Johns 1996
Mediophyceae	*Odontella aurita*		Guihéneuf et al. 2010
			Pulz and Gross 2004
Chlorophyta (green algae)			
Prasinophyceae	*Ostreococcus tauri*	EPA, DHA	Wagner et al. 2010
	Micromonas pusilla *Pyramimonas cordata*		Dunstan et al. 1992
Trebouxiophyceae	*Parietochloris incisa*	ARA	Bigogno et al. 2002a
	P. incisa (Δ5 desaturase mutant)	DGLA	Iskandarov et al. 2011
Cryptophyta			
Cryptophyceae	*Chroomonas salina*	DHA	Henderson and Mackinlay 1992
Dinoflagellata			
Dinophyceae	*Pyrocystis fusiformis*, *P. lunula*, *P. noctiluca*	EPA, OPA, SDA	Leblond et al. 2010
Eustigmatophyta			
Eustigmatophyceae	*Nannochloropsis* sp. *N. salina, N. oculata*	EPA	Volkman et al. 1993; Sukenik 1999
	Monodus subterraneus	EPA	Cohen 1994
			Khozin-Goldberg et al. 2002a
Haptophyta			
Prymnesiophyceae	*Isochrysis galbana*	SDA, EPA, DHA	Molina Grima et al. 1992
			Qi et al. 2002
	Emiliania huxleyi	DHA, OPA	Sayanova et al. 2011
Pavlovophyceae	*Pavlova lutheri*	EPA, DHA, SDA	Volkman et al. 1991
			Kato et al. 1995
			Meireles et al. 2003
Rhodophyta			
Porphyridiophyceae	*Porphyridium cruentum*	ARA, EPA	Cohen 1999
			Khozin et al. 1997
Xanthophyta			
Xanthophyceae	*Trachydiscus minutus*	EPA	Řezanka et al. 2010

Classification according to Guiry and Guiry 2011

The diversity of microalgal classes with respect to LC-PUFA production is illustrated in Table 1; the ω-3 LC-PUFA EPA and DHA are abundant in representatives of different classes dwelling in marine environments. This capacity is utilized in aquaculture nutrition and fish farming where many microalgal species are routinely cultivated to provide fish with EPA and DHA (Benemann 1992; Lavens and Sorgeloos 1996). However, ω-6 LC-PUFA are relatively rare in algae, appearing mainly as precursors in the biosynthesis of EPA; moreover, in most marine species, they do not account for more than a few percent of total fatty acids (Thompson 1996). ARA is also rare in the lipids of freshwater algae; however, in the chlorophyte *Parietochloris incisa* (Bigogno et al. 2002a,b), ARA reaches about 60% of total fatty acids under conditions of nitrogen starvation (Khozin-Goldberg et al. 2002a). High contents of DGLA are not found in any alga, unless it has undergone genetic manipulation, such as the ARA-deficient Δ5 desaturase mutant of *P. incisa* obtained by chemical mutagenesis (Iskandarov et al. 2011; Solovchenko et al. 2010).

Many LC-PUFA-producing microalgae, e.g., species of red algae, diatoms, and eustigmatophytes, contain low levels of C_{18} fatty acid precursors. However, in other species, LC-PUFAs occur along with C_{18} PUFA, as is typical in higher plants (Bigogno et al. 2002a). Aside from the C_{18} PUFA 18:3ω-3 and 18:3ω-6, representatives of certain microalgal classes contain the highly unsaturated ω-

3 C_{18} PUFA octadecatrienoic acid (18:4ω-3, alternatively steriadonic acid, SDA) and octadecapentaenoic acid (OPA, 18:5ω-3). The presence of highly unsaturated C_{18} PUFA appears to be typical to dinoflagellates of the genus *Pyrocystis* (Leblond et al. 2010) and to haptophytes such as, for example, the coccolithophore *Emiliania huxleyi* (Sayanova et al. 2011), where these fatty acids occur concomitantly with ω-3 LC-PUFA.

Biosynthesis of LC-PUFA in microalgae

The biosynthesis of LC-PUFA from C_{18} fatty acids in microalgae may follow different routes (Fig. 1), utilizing iterative desaturation and elongation steps. Fatty acid desaturases catalyze the introduction of *cis* double bonds at specific positions in the fatty acid chain. PUFA-specific elongation complex mediates a two-carbon extension of the acyl chain by condensation of malonyl-CoA to an existing acyl-CoA moiety (Cinti et al. 1992; Meyer et al. 2004). In general, the LC-PUFA biosynthesis pathways in the endoplasmic reticulum (ER) are initiated by Δ12 desaturation of the chloroplast-derived oleic acid (OA, 18 : $1^{\Delta 9}$, ω-9), producing LA (18 :$2^{\Delta 9,12}$, ω-6). Subsequently, LA may be further desaturated by a Δ15 (ω-3) desaturase, generating ALA (18 : $3^{\Delta 9,12,15}$, ω-3) (Fig. 1). These fatty acids are further converted via the common ω-6 and ω-3 pathways, which are initiated with the Δ6 desaturation of LA or ALA, respectively. Alternative pathways that initiate with a Δ9-specific elongation of LA or ALA to eicosadienoic acid (20 : $2^{\Delta 11,14}$, ω-6) or eicosatrienoic acid (20 : $3^{\Delta 11,14,17}$, ω-3), respectively, followed by sequential Δ8 and Δ5 desaturations, exist in some microalgae, such as the haptophytes *Isochrysis galbana* (Qi et al. 2002), *Pavlova salina* (Zhou et al. 2007), and *E. huxleyi* (Sayanova et al. 2011), and the freshwater euglenophyte *Euglena gracilis* (Wallis and Browse 1999). In the ω-6 and ω-3 pathways, the products of Δ6-desaturated products of LA and ALA respectively, further undergo Δ6 PUFA elongation and Δ5 desaturation via respective intermediates, ultimately yielding ARA or EPA (Abbadi et al. 2004; Cohen et al. 1992; Meyer et al. 2004). As can be seen in Fig. 1, all pathways share some C_{20} intermediates. In the diatom *Phaeodactylum tricornutum*, both the ω-3 and ω-6 pathways are active (Arao et al. 1994), and their intermediates contribute to the biosynthesis of EPA (Domergue et al. 2002). However, one pathway may sometimes dominate over the other, for instance, the major ω-6 pathway, which has been suggested to operate in the rhodophyte *Porphyridium cruentum* (Khozin et al. 1997), the freshwater chlorophyte *P. incisa* (Bigogno et al. 2002c; Iskandarov et al. 2009, 2010) and two eustigmatophytes, the freshwater *Monodus subterraneus* (Khozin-Goldberg et al. 2002b), and the marine *Nannochloropsis* sp. (Schneider and Roessler 1994; Sukenik 1999). *P. incisa* represents a rare case in which ARA is the major and almost ultimate product of the extraplastidial lipid-linked biosynthesis pathway, which is further accumulated in the reserve lipids, the triacylglycerols (TAG) (Bigogno et al. 2002c). Conversion of ARA to EPA occurs at a very low rate in this alga, but can be increased at low temperature. C_{20} ω-3 desaturation is mediated by Δ17 desaturase, which desaturates ARA to EPA, as in the EPA-

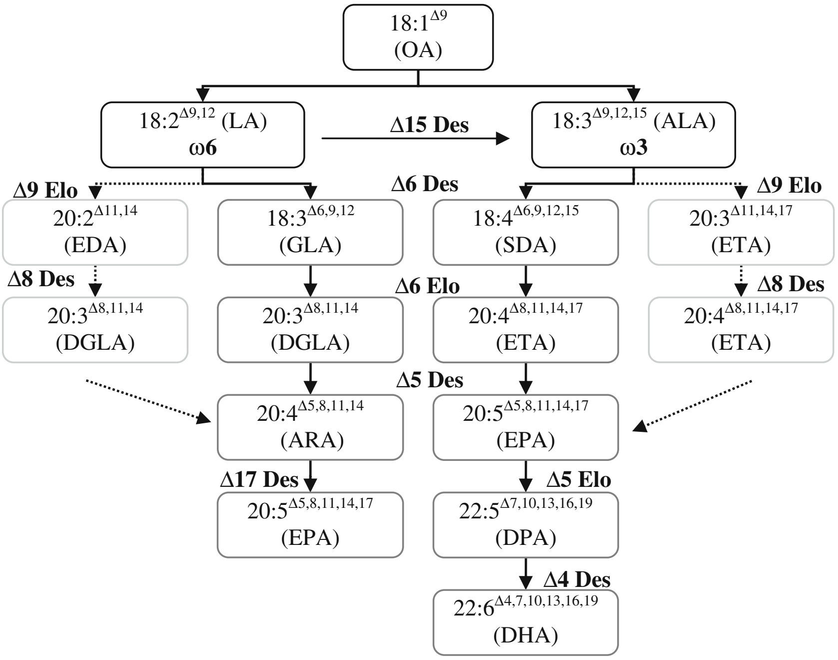

Fig. 1 Pathways for the biosynthesis of LC-PUFA in microalgae

producing *Nannochloropsis* sp. (Schneider and Roessler 1994), *M. subterraneus* (Khozin-Goldberg et al. 2002b), and *P. cruentum (*Khozin et al. 1997). The final step of EPA biosynthesis, however, does not necessarily occur in the ER. ARA can be relocated to the plastid, probably by a mechanism similar to trafficking of the ER-produced ALA in higher plants (Benning 2009). Radiolabeling pulse-chase experiments allowed us to suggest that in the red microalga *P. cruentum*, ARA is exported to the chloroplast where the final desaturation step of EPA biosynthesis occurs, involving membrane lipids (Khozin et al. 1997). In *Nannochloropsis* sp. and *M. subterraneus*, however, EPA is produced in the ER and is further exported to the plastid and incorporated into the galactolipids (Khozin-Goldberg et al. 2002b; Sukenik 1999). The Δ17 (ω-3) desaturases of these microalgae have not yet been cloned or characterized, and their functional role in LC-PUFA biosynthesis therefore awaits confirmation at the molecular level.

In the C_{22} LC-PUFA-producing microalgae, biosynthesis of DHA in the extraplastidial compartment is mediated by C_{20}-specific two-carbon chain elongation of EPA to docosapentaenoic acid, 22:5ω-3 (DPA, 22:5ω-3), followed by Δ4 desaturation (Fig. 1). Δ4 desaturases have been cloned and characterized from various microalgae (Meyer et al. 2003; Pereira et al. 2004; Tonon et al. 2002; Zhou et al. 2007). This relatively recently discovered route is distinct from the peroxisomal Sprecher pathway of DHA biosynthesis in mammals (Sprecher 2000) and the anaerobic polyketide synthase (PKS) pathway discovered in some heterotrophic marine eukaryotes of the Thraustochytriaceae family (Metz et al. 2001). The PKS pathway does not require aerobic desaturation, while the double bonds are introduced during the process of fatty acid synthesis.

Desaturases engaged in LC-PUFA biosynthesis (Δ4, Δ5, Δ6, and Δ8) are non-heme "front-end" membrane-bound enzymes, which introduce a new double bond between the pre-existing double bond and the carboxyl end of the fatty acid (Sayanova and Napier 2004). Typical features of front-end desaturases are the presence of three conserved histidine-rich motifs (boxes) and an N-terminal cytochrome b5-fused domain, which serves as an electron donor. Since various desaturases involved in LC-PUFA biosynthesis share similar structural features, investigations of their predicted functions by functional expression and characterization is an essential step in their proper designation (Sayanova et al. 2011). For example, a recent study describes the identification and functional characterization of the genes involved in the biosynthesis of ω-3 LC-PUFA in the coccolithophore *E. huxleyi* (Sayanova et al. 2011). The authors searched a draft genome (v.1.1 release) of this microalga for the candidate desaturases and elongases involved in LC-PUFA biosynthesis. Their functional characterization in a yeast expression system revealed that *E. huxleyi* synthesizes DHA via the alternative pathway, which is initiated by a C_{18} Δ9 elongase (Fig. 1). It was suggested that the biosynthesis of the highly unsaturated Δ6-desaturated C_{18} fatty acids SDA and OPA is metabolically distinct from DHA biosynthesis. This was supported by two pieces of evidence: the absence of OPA acyl-CoA from the acyl-CoA pool and the high abundance of both SDA and OPA in chloroplast membrane lipids. The interest in elucidating SDA biosynthesis is driven by the fact that this fatty acid shares many of the health benefits of EPA, and its dietary intake improves EPA levels.

Until recently, lipid-linked desaturation was considered to be the main route operating in the ER of microalgae, similar to higher plants. In this route, fatty acid substrates are attached to phospholipids of the ER, such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE), or to the betaine lipid, diacylglyceroltrimethylhomoserine (DGTS), in microalgae containing this non-phosphorus polar lipid. For example, in the ARA-producing *P. incisa*, it was suggested that three extraplastidial lipids are involved in various steps of ARA biosynthesis: PC and DGTS are involved in the Δ12, and subsequently Δ6 desaturations, whereas PE along with PC are the suggested major substrates for the Δ5 desaturation of 20:3ω-6 to 20:4ω-6 (Bigogno et al. 2002c). The lipid-linked nature of the Δ5 and Δ6 desaturases was confirmed by functional expression of the cloned enzymes in the yeast *Saccharomyces cerevisiae* (Iskandarov et al. 2010). The last few years have been characterized by outstanding progress in sequencing genomes of microalgae, also due to the introduction of new-generation sequencing technologies. Two recent reviews provide a detailed summary of the available information on nuclear, mitochondrion, and chloroplast genomes and EST projects of versatile microalgal species (refer to Lü et al. 2011; Tirichine and Bowler 2011). Thanks to the genome annotations of several LC-PUFA-producing microalgal species, functional analysis of several novel microalgal desaturases and elongases has been performed by various research groups (e.g., Domergue et al. 2002, 2005; Tonon et al. 2005; Sayanova et al. 2011). It appears that some algal Δ6 and Δ5 desaturases act on CoA-activated PUFA, similar to mammalian front-end desaturases (Domergue et al. 2005; Hoffmann et al. 2008; Petrie et al. 2010a). This feature offers great promise in plant biotechnology for the engineering of oilseed plants to produce LC-PUFA, as well as in the metabolic engineering of microalgae. The use of acyl-CoA-dependent enzymes with the correct substrate specificities may eliminate the requirement for the rate-limiting acyl exchange with membrane polar lipids and thus avoid "substrate dichotomy" for lipid-linked desaturases and PUFA elongases, utilizing acyl-CoA substrates (for further information, see, Napier and Graham 2010; Petrie et al. 2010b; Venegas-

Calerón et al. 2010). Furthermore, many of the recently cloned microalgal acyl-CoA-dependent desaturases have been shown to be highly specific for the ω-3 substrates when expressed in a recombinant system. This preference appears to be beneficial for the metabolic engineering of ω-3 LC-PUFA (EPA and DHA) biosynthesis in higher plants.

Commercial prospects

As we have seen, photosynthetic microalgae represent an abundant and largely untapped resource for LC-PUFA. Commercial amounts of these fatty acids are currently produced by fermentation of several heterotrophic single-cell organisms, the microalga *C. cohnii* (Kyle 1996, 2001; Ratledge 1998), and the marine protists belonging to the phylum Labyrinthulomycota, the Labyrinthulales, such as *Schizochytrium* sp. (Hauvermale et al. 2006; Lippmeier et al. 2009), and the Thraustochytriales, such as *Thraustochytrium*, are sources for ω-3 LC-PUFA (Raghukumar 2008; Singh and Ward 1996). According to some estimates, heterotrophic LC-PUFA production by microalgae was valued at $195 million in 2004, with the ω-3 PUFA market increasing at an average growth rate of 8% from 2004 to 2010 (http://www.frost.com/prod/servlet/report-brochure.pag?id=B329-01-00-00-00).

As for photosynthetic LC-PUFA-producing microalgae, numerous species (*Isochrysis*, *Chaetoceros*, *Nannochloropsis*, *Phaedoactylum*, and *Pavlova*) are cultivated in the aquaculture industry at relatively low cell densities, mainly for the enrichment of microscopic zooplankton and fish juvenile stages (Benemann 1992; Reitan et al. 1997; Seto et al. 1992). Economically feasible cultivation of photosynthetic microalgae for large-scale production of LC-PUFA for human nutrition requires substantial advances in photobiotechnology and breakthrough solutions for several technological and biochemical bottlenecks; these should result in reduced costs of biomass production and lipid/LC-PUFA extraction.

Ratledge and Cohen (2008) suggested that current prospects in microalgal biotechnology should focus on algae as sources of LC-PUFA rather than for biodiesel production. As a result, reduced prices could be expected to result from the utilization of less expensive cultivation technologies, such as open ponds and low-cost photobioreactors, and the exploitation of robust, fast-growing algae that can withstand predatory organisms, contaminating bacteria, fungi, or competing algae, while simultaneously attaining high LC-PUFA contents (Ratledge and Cohen 2008). In fact, the economical production of algal LC-PUFA is likely to become more realistic in the coming years, given the research emphasis on advances in microalgal technology in the pursuit of renewable oil production by microalgae as an alternative feedstock for biodiesel (Radakovits et al. 2010; Scott et al. 2010). Current R&D is centered on creating high-efficiency technologies for photosynthetic algal cultivation to reduce energy input, cost of construction, operation, and harvesting, which utilize inexpensive CO_2 resources (such as a flue gas), non-arable lands, and saline water resources of limited alternative use (reviewed in Morweiser et al. 2010; Stephens et al. 2010; Tredici 2010; Wijffels and Barbosa 2010). Physiological studies and genetic engineering approaches are directed to enhancing growth performance and increasing or modifying lipid content and fatty acid composition (Radakovits et al. 2010). Genetic engineering is also aimed at utilizing molecular tools to create strains capable of efficiently capturing light in dense cultures and of resisting contamination. Global production of microalgae is growing quickly due to its anticipated commercial potential and the ecological significance of renewable oil generation. This is perhaps best reflected in the large number of commercial ventures (summarized in Lü et al. 2011) and international events devoted to microalgal cultivation and commercial product development (e.g. http://www.algaeurope.eu; http://www.biofuelstp.eu/algae.htm). As a whole, it is believed that this effort will accelerate the commercialization of algae as an oil resource to within 10 or 15 years (Wijffels and Barbosa 2010). However, another opinion exists based on technology and engineering assessments, which states that even with relatively favorable process assumptions, oil production for biodiesel using microalgae will be expensive (Lundquist et al. 2010). Biorefinery is thus seen as an approach to maximizing the exploitation of valuable algal components after extraction of their oil, suitable for biodiesel production, with the aim of increasing commercial potential. The potential of microalgae to synthesize valuable products, such as LC-PUFA, in addition to their use for energy, should be integrated into a production concept (Morweiser et al. 2010).

At present, few photosynthetic species of green microalgae are mainly cultivated on a large scale for the production of high-value constituents for the human health food market (Borowitzka 1988; Pulz and Gross 2004; Spolaore et al. 2006). Species such as *Chlorella vulgaris* (a source of proteins, vitamins, and biologically active compounds) are cultured by Roquette Klötze GmbH & Co. KG, Germany; *Dunaliella salina* (Cognis, Australia; NBT, Japan) and *Haematococcus pluvialis* (e.g. Algatech, Israel, Cyanotech, USA) are cultivated on a large scale for carotenoid production—β-carotene and astaxanthin, respectively. Total world production of dry algal biomass for these species is estimated at about 10,000 t per year (http://www.fao.org/bioenergy/aquaticbiofuels/knowledge/en/).

Due to its clinically proven beneficial health effects, EPA has become a promising target for microalgal biotechnology. In recent years, several photosynthetic EPA-producing microalgae, such as the eustigmatophytes of the genus *Nannochloropsis* (*Nannochloropsis oculata*, *Nannochloropsis salina*, and *Nannochloropsis gaditana*) and the diatom *Odontella aurita*, have attracted significant attention. Several companies worldwide are engaged in the cultivation of algae from the genus *Nannochloropsis* for EPA, e.g., Sembiotic (Israel), LGem (The Netherlands), and Nekton-Algafuel (Portugal), among others. These microalgae, mainly planktonic marine species, are rich in EPA (Hodgson et al. 1991; Sukenik 1999; Volkman et al. 1993) and hold promise as a potential source of this constituent for the human health market. *Nannochloropsis* is grown in both open pond systems and photobioreactors (Boussiba et al. 1987; Richmond et al. 2003; Rodolfi et al. 2009; Sukenik 1999; Sukenik et al. 2009). Numerous studies are devoted to the optimization of its biomass and EPA productivity (Chini Zittelli et al. 1999; Pal et al. 2011; Zou et al. 2000); the maximum reported values for EPA content of biomass is about 5% of dry weight. The interest in *Nannochloropsis* cultivation is driven by its high EPA percentage of total fatty acids (up to 35%) and absence of DHA, thus presenting a good source of a single ω-3 LC-PUFA for dietary purposes. The ω-6 LC-PUFA, ARA, amounts to only a few percent of total fatty acids in this alga, and the other major fatty acids are C_{16} fatty acids, palmitic acid (16:0), and palmitoleic acid (16:1). It is commonly accepted that a desirable dietary source should contain LC-PUFA in TAG (oil). In general, most microalgae contain LC-PUFA as constituents of their polar lipids, while the accumulation of LC-PUFA in TAG is very rare (Cohen and Khozin-Goldberg 2005). Despite the fact that EPA in *Nannochloropsis* is mainly attached to the chloroplast membrane lipids, dietary feeding with its biomass has been shown to be effective at increasing levels of EPA in the blood, plasma, and muscle of rats and poultry (Nitsan et al. 1999; Sukenik et al. 1994). Accumulation of TAG, consisting of saturated and monounsaturated acyl moieties, occurs in response to environmental stresses such as nitrogen starvation, salinity, or high light (Pal et al. 2011). Cultivated under conditions of nitrogen starvation, *Nannochloropsis* is a potent source of saturated and monounsaturated oils and thus holds promise for biodiesel production (Rodolfi et al. 2009). However, EPA percentage in fatty acids of TAG may account for a few percent when TAG accumulation in *Nannochloropsis* sp. is induced by increasing light intensity on nitrogen-replete medium (Pal et al. 2011).

Another commercial source for phototrophic EPA is the diatom *O. aurita*, which contains 27–28% EPA out of total lipids along with 4% DHA (Guihéneuf et al. 2010). It is cultivated on a commercial scale by Innovalg Co. in France and is approved as a human food supplement (AFSSA 2001, AFSSA Saisine no. 2001-SA-0082; CE 285/97). An emerging algal species is described by Řezanka et al. (2010), who reported that *Trachydiscus minutes* produces EPA under conditions of nutrient starvation and, most importantly, accumulates it in TAG.

Aurora Algae Inc. (http://www.aurorainc.com) announced a novel algal-based product containing 60% EPA. Although the strain was not specified, the company claims that it has developed the industry's first commercial-scale photosynthetic platform for sustainable, alga-based product development using proprietary algal strains. The production process makes use of arid land, seawater, and CO_2 captured from industrial emitters.

As already mentioned, microalgae accumulate TAG under stress conditions, such as nitrogen limitation, salinity, or high light intensity (Roessler 1990). TAGs in most algae contain mostly saturated and monounsaturated fatty acids rather than LC-PUFA (Cohen and Khozin-Goldberg 2005), while LC-PUFA-enriched TAG is a desirable form for dietary intake and ingestion. The ability to accumulate large amounts of LC-PUFA in TAG would be a desirable feature for microalgae destined for use as an economically feasible source of LC-PUFA and in the development of biotechnological processes as well, due to the relative ease of oil extractions with non-polar non-toxic solvents. It should be noted, however, that algal cells are often surrounded by a thick cell wall, which requires the use of sophisticated methods for cell breakage. A rare example of LC-PUFA-enriched TAG is the ARA-containing TAG of the freshwater green alga *P. incisa* (Bigogno et al. 2002a,b; Khozin-Goldberg et al. 2002a). This alga accumulates up to 60% ARA in its TAG upon cultivation under nitrogen-starvation conditions and represents a potential photosynthetic source for ARA (Solovchenko et al. 2008). Its mutant, deficient in ARA due to a non-sense mutation in the Δ5 desaturase gene, produces DGLA at up to 12–14% of dry weight. DGLA normally occurs only as an intermediate in the biosynthesis of ARA, but is not appreciably accumulated in any organism. The mutant produces trace amounts of ARA and high contents of DGLA (up to 35% of total fatty acids), making the mutant a potential source for the production of this pharmaceutically important LC-PUFA (Iskandarov et al. 2011; Solovchenko et al. 2010).

In conclusion, LC-PUFA production by photosynthetic microalgae holds substantial promise. As already proposed by Ratledge in 1998, the future appears to be bright for the exploitation of marine microorganisms, including microalgae, for the production of key LC-PUFA. The market for ω-3 LC-PUFA is growing rapidly due to increasing global awareness of their health-beneficial properties. This global scope has attracted industrial alga-growing companies and marketers. Exploitation of these organisms' high potential can be achieved through advances in technology and strain improvements stemming from a thorough knowledge of algal physiology and lipid biochemistry, which will enable consistent and sustainable biomass production. Metabolic engineering of photosynthetic microalgae for LC-PUFA production should play a central role in the development of a cost-effective clean alternative to fish oil. This would be supported by the establishment of genetic transformation systems for additional biotechnologically important species to enable manipulation of LC-PUFA biosynthesis.

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