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(54) Title: NOVEL FATTY ACID DESATURASES AND ELONGASES AND USES THEREOF

(57) Abstract: The invention provides isolated nucleic acid molecules which encode novel fatty acid desaturases and elongases from the organism *Emilia huxleyi*. The invention also provides recombinant expression vectors containing desaturase or elongase nucleic acid molecules, host cells into which the expression vectors have been introduced, and methods for large-scale production of long chain polyunsaturated fatty acids (LCPUFAs), e.g. arachidonic acid (ARA), eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA).

NOVEL FATTY ACID DESATURASES AND ELONGASES AND USES THEREOF

The invention in principle pertains to the field of recombinant manufacture of fatty acids. It provides nucleic acid molecules which encode novel fatty acid desaturases and elongases.

5 The invention also provides recombinant expression vectors containing desaturase and elongase nucleic acid molecules, host cells into which the expression vectors have been introduced, and methods for large-scale production of long chain polyunsaturated fatty acids (LCPUFAs), e.g. arachidonic acid (ARA), eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA).

10 Fatty acids are carboxylic acids with long-chain hydrocarbon side groups that play a fundamental role in many biological processes. Fatty acids are rarely found free in nature but, rather, occur in esterified form as the major component of lipids. As such, lipids/ fatty acids are sources of energy (e.g., β -oxidation). In addition, lipids/ fatty acids are an integral 15 part of cell membranes and, therefore, are indispensable for processing biological or biochemical information.

Fatty acids can be divided into two groups: saturated fatty acids formed of single carbon bonds and the unsaturated fatty acids which contain one or more carbon double bonds in 20 cis-configuration. Unsaturated fatty acids are produced by terminal desaturases that belong to the class of nonheme-iron enzymes. Each of these enzymes are part of an electron-transport system that contains two other proteins, namely cytochrome b_5 and NADH-cytochrome b_5 reductase. Specifically, such enzymes catalyze the formation of double bonds between the carbon atoms of a fatty acid molecule, for example, by catalyzing the 25 oxygen-dependent dehydrogenation of fatty acids (Sperling et al., 2003). Human and other mammals have a limited spectrum of desaturases that are required for the formation of particular double bonds in unsaturated fatty acids and thus, have a limited capacity for synthesizing essential fatty acids, e.g., long chain polyunsaturated fatty acids (LCPUFAs). Thus, humans have to take up some fatty acids through their diet. Such essential fatty acids 30 include, for example, linoleic acid (C18:2), linolenic acid (C18:3). In contrast, insects, microorganisms and plants are able to synthesize a much larger variety of unsaturated fatty acids and their derivatives. Indeed, the biosynthesis of fatty acids is a major activity of plants and microorganisms.

35 Long chain polyunsaturated fatty acids (LCPUFAs) such as docosahexaenoic acid (DHA, 22:6(4,7,10,13,16,19)) are essential components of cell membranes of various tissues and organelles in mammals (nerve, retina, brain and immune cells). For example, over 30% of fatty acids in brain phospholipid are 22:6 (n-3) and 20:4 (n-6) (Crawford, M.A., et al., (1997) Am. J. Clin. Nutr. 66:1032S-1041S). In retina, DHA accounts for more than 60% of the total 40 fatty acids in the rod outer segment, the photosensitive part of the photoreceptor cell (Giusto, N.M., et al. (2000) Prog. Lipid Res. 39:315-391). Clinical studies have shown that DHA is essential for the growth and development of the brain in infants, and for maintenance of normal brain function in adults (Martinetz, M. (1992) J. Pediatr.

120:S129-S138). DHA also has significant effects on photoreceptor function involved in the signal transduction process, rhodopsin activation, and rod and cone development (Giusto, N.M., et al. (2000) Prog. Lipid Res. 39:315-391). In addition, some positive effects of DHA were also found on diseases such as hypertension, arthritis, atherosclerosis, depression, 5 thrombosis and cancers (Horrocks, L.A. and Yeo, Y.K. (1999) Pharmacol. Res. 40:21 1-215). Therefore, appropriate dietary supply of the fatty acid is important for human health. Because such fatty acids cannot be efficiently synthesized by infants, young children and senior citizens, it is particularly important for these individuals to adequately intake these fatty acids from the diet (Spector, A.A. (1999) Lipids 34:S1-S3).

10

Currently the major sources of DHA are oils from fish and algae. Fish oil is a major and traditional source for this fatty acid, however, it is usually oxidized by the time it is sold. In addition, the supply of fish oil is highly variable, particularly in view of the shrinking fish populations. Moreover, the algal source of oil is expensive due to low yield and the high 15 costs of extraction.

15

EPA and ARA are both $\Delta 5$ essential fatty acids. They form a unique class of food and feed constituents for humans and animals. EPA belongs to the n-3 series with five double bonds in the acyl chain. EPA is found in marine food and is abundant in oily fish from North 20 Atlantic. ARA belongs to the n-6 series with four double bonds. The lack of a double bond in the co-3 position confers on ARA different properties than those found in EPA. The eicosanoids produced from AA have strong inflammatory and platelet aggregating properties, whereas those derived from EPA have anti-inflammatory and anti-platelet 25 aggregating properties. ARA can be obtained from some foods such as meat, fish and eggs, but the concentration is low.

25

Gamma-linolenic acid (GLA) is another essential fatty acid found in mammals. GLA is the metabolic intermediate for very long chain n-6 fatty acids and for various active molecules. In mammals, formation of long chain polyunsaturated fatty acids is rate-limited by $\Delta 6$ 30 desaturation. Many physiological and pathological conditions such as aging, stress, diabetes, eczema, and some infections have been shown to depress the $\Delta 6$ desaturation step. In addition, GLA is readily catabolized from the oxidation and rapid cell division associated with certain disorders, e.g., cancer or inflammation. Therefore, dietary 35 supplementation with GLA can reduce the risks of these disorders. Clinical studies have shown that dietary supplementation with GLA is effective in treating some pathological conditions such as atopic eczema, premenstrual syndrome, diabetes, hypercholesterolemia, and inflammatory and cardiovascular disorders.

40

A large number of beneficial health effects have been shown for DHA or mixtures of EPA/DHA. DHA is a n-3 very long chain fatty acid with six double bonds.

Although biotechnology offers an attractive route for the production of specialty fatty acids,

current techniques fail to provide an efficient means for the large scale production of unsaturated fatty acids. Accordingly, there exists a need for an improved and efficient method of producing unsaturated fatty acids, such as DHA, EPA and ARA.

- 5 Thus, the present invention relates to a polynucleotide comprising a nucleic acid sequence selected from the group consisting of:
- a) a nucleic acid sequence having a nucleotide sequence as shown in SEQ ID NOs: 1, 3, 5, 7 or 9;
 - b) a nucleic acid sequence encoding a polypeptide having an amino acid sequence as 10 shown in SEQ ID NOs: 2, 4, 6, 8 or 10;
 - c) a nucleic acid sequence being at least 70% identical to the nucleic acid sequence of a) or b), wherein said nucleic acid sequence encodes a polypeptide having desaturase or elongase activity;
 - d) a nucleic acid sequence encoding a polypeptide having desaturase or elongase 15 activity and having an amino acid sequence which is at least 82% identical to the amino acid sequence of any one of a) to c); and
 - e) a nucleic acid sequence which is capable of hybridizing under stringent conditions to any one of a) to d), wherein said nucleic acid sequence encodes a polypeptide having desaturase or elongase activity.

20 The term " polynucleotide" as used in accordance with the present invention relates to a polynucleotide comprising a nucleic acid sequence which encodes a polypeptide having desaturase or elongase activity. Preferably, the polypeptide encoded by the polynucleotide of the present invention having desaturase or elongase activity upon expression in a plant 25 shall be capable of increasing the amount of PUFA and, in particular, LCPUFA in, e.g., seed oils or the entire plant or parts thereof. Such an increase is, preferably, statistically significant when compared to a LCPUFA producing transgenic control plant which expresses the the present state of the art set of desaturases and elongases requiered for LCPUFA synthesis but does not express the polynucleotide of the present invention.

30 Whether an increase is significant can be determined by statistical tests well known in the art including, e.g., Student's t-test. More preferably, the increase is an increase of the amount of triglycerides containing LCPUFA of at least 5%, at least 10%, at least 15%, at least 20% or at least 30% compared to the said control. Preferably, the LCPUFA referred to before is a polyunsaturated fatty acid having a C-20 or C-22 fatty acid body, more 35 preferably, ARA, EPA or DHA. Suitable assays for measuring the activities mentioned before are described in the accompanying Examples.

40 The term " desaturase" or " elongase" as used herein refers to the activity of a desaturase, introducing a double bond into the carbon chain of a fatty acid, preferably into fatty acids with 18, 20 or 22 carbon molecules, or an elongase, introducing two carbon molecules into the carbon chain of a fatty acid, preferably into fatty acids with 18, 20 or 22 carbon molecules

More preferably, polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 1, 3, 5, 7 or 9 encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 2, 4, 6, 8 or 10 or variants thereof, preferably, exhibit desaturase or elongase activity.

5

Polynucleotides encoding a polypeptide having desaturase or elongase activity as specified above has been obtained in accordance with the present invention, preferably, from *Emiliana huxleyi*. However, orthologs, paralogs or other homologs may be identified from other species. Preferably, they are obtained from plants such as algae, for example

10 *Isochrysis*, *Mantoniella*, *Ostreococcus* or *Cryptocodonium*, algae/diatoms such as *Phaeodactylum*, *Thalassiosira* or *Thraustochytrium*, mosses such as *Physcomitrella* or *Ceratodon*, or higher plants such as the *Primulaceae* such as *Aleuritia*, *Calendula stellata*, *Osteospermum spinescens* or *Osteospermum hyoseroides*, microorganisms such as fungi, such as *Aspergillus*, *Phytophthora*, *Entomophthora*, *Mucor* or *Mortierella*, bacteria such as

15 *Shewanella*, yeasts or animals. Preferred animals are nematodes such as *Caenorhabditis*, insects or vertebrates. Among the vertebrates, the nucleic acid molecules may, preferably, be derived from *Euteleostomi*, *Actinopterygii*; *Neopterygii*; *Teleostei*; *Euteleostei*, *Protacanthopterygii*, *Salmoniformes*; *Salmonidae* or *Oncorhynchus*, more preferably, from the order of the *Salmoniformes*, most preferably, the family of the *Salmonidae*, such as the

20 genus *Salmo*, for example from the genera and species *Oncorhynchus mykiss*, *Trutta trutta* or *Salmo trutta fario*. Moreover, the nucleic acid molecules may be obtained from the diatoms such as the genera *Thallasiosira* or *Phaeodactylum*.

Thus, the term " polynucleotide" as used in accordance with the present invention further encompasses variants of the aforementioned specific polynucleotides representing orthologs, paralogs or other homologs of the polynucleotide of the present invention. Moreover, variants of the polynucleotide of the present invention also include artificially generated muteins. Said muteins include, e.g., enzymes which are generated by mutagenesis techniques and which exhibit improved or altered substrate specificity, or codon optimized polynucleotides. The polynucleotide variants, preferably, comprise a nucleic acid sequence characterized in that the sequence can be derived from the aforementioned specific nucleic acid sequences shown in any one of SEQ ID NOs: 1, 3, 5, 7 or 9 or by a polynucleotide encoding a polypeptide having an amino acid sequence as shown in any one of SEQ ID NOs: 2, 4, 6, 8 or 10 by at least one nucleotide substitution, addition and/or deletion, whereby the variant nucleic acid sequence shall still encode a polypeptide having a desaturase or elongase activity as specified above. Variants also encompass polynucleotides comprising a nucleic acid sequence which is capable of hybridizing to the aforementioned specific nucleic acid sequences, preferably, under stringent hybridization conditions. These stringent conditions are known to the skilled worker and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred example for stringent hybridization conditions are hybridization conditions in 6 x sodium chloride/sodium citrate (= SSC) at approximately

45°C, followed by one or more wash steps in 0.2 x SSC, 0.1 % SDS at 50 to 65°C. The skilled worker knows that these hybridization conditions differ depending on the type of nucleic acid and, for example when organic solvents are present, with regard to the temperature and concentration of the buffer. For example, under " standard hybridization 5 conditions" the temperature differs depending on the type of nucleic acid between 42°C and 58°C in aqueous buffer with a concentration of 0.1 to 5 x SSC (pH 7.2). If organic solvent is present in the abovementioned buffer, for example 50% formamide, the temperature under standard conditions is approximately 42°C. The hybridization conditions for DNA: DNA hybrids are, preferably, 0.1 x SSC and 20°C to 45°C, preferably between 10 30°C and 45°C. The hybridization conditions for DNA:RNA hybrids are, preferably, 0.1 x SSC and 30°C to 55°C, preferably between 45°C and 55°C. The abovementioned hybridization temperatures are determined for example for a nucleic acid with approximately 100 bp (= base pairs) in length and a G + C content of 50% in the absence of formamide. The skilled worker knows how to determine the hybridization conditions required by 15 referring to textbooks such as the textbook mentioned above, or the following textbooks: Sambrook et al., "Molecular Cloning" , Cold Spring Harbor Laboratory, 1989; Hames and Higgins (Ed.) 1985, " Nucleic Acids Hybridization: A Practical Approach" , IRL Press at Oxford University Press, Oxford; Brown (Ed.) 1991 , "Essential Molecular Biology: A Practical Approach" , IRL Press at Oxford University Press, Oxford. Alternatively, 20 polynucleotide variants are obtainable by PCR-based techniques such as mixed oligonucleotide primer- based amplification of DNA, i.e. using degenerated primers against conserved domains of the polypeptides of the present invention. Conserved domains of the polypeptide of the present invention may be identified by a sequence comparison of the nucleic acid sequences of the polynucleotides or the amino acid sequences of the 25 polypeptides of the present invention. Oligonucleotides suitable as PCR primers as well as suitable PCR conditions are described in the accompanying Examples. As a template, DNA or cDNA from bacteria, fungi, plants or animals may be used. Further, variants include polynucleotides comprising nucleic acid sequences which are at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 30 90%, at least 95%, at least 98% or at least 99% identical to the nucleic acid sequences shown in any one of SEQ ID NOs: 1, 3, 5, 7 or 9, preferably, encoding polypeptides retaining a desaturase or elongase activity as specified above. Moreover, also encompassed are polynucleotides which comprise nucleic acid sequences encoding a polypeptide having an amino acid sequences which are at least 50%, at least 55%, at least 35 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequences shown in any one of SEQ ID NOs: 2, 4, 6, 8 or 10, wherein the polypeptide, preferably, retains desaturase or elongase activity as specified above. The percent identity values are, preferably, calculated over the entire amino acid or nucleic acid sequence region. A series of programs 40 based on a variety of algorithms is available to the skilled worker for comparing different sequences. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch algorithm (Needleman 1970, J.

Mol. Biol. (48):444-453) which has been incorporated into the needle program in the EMBOSS software package (EMBOSS: The European Molecular Biology Open Software Suite, Rice,P., Longden,!., and Bleasby,A, Trends in Genetics 16(6), 276-277, 2000), using either a BLOSUM 45 or PAM250 scoring matrix for distantly related proteins, or either a

5 BLOSUM 62 or PAM160 scoring matrix for closer related proteins, and a gap opening penalty of 16, 14, 12, 10, 8, 6, or 4 and a gap entension pentalty of 0.5, 1, 2, 3, 4, 5, or 6. Guides for local installation of the EMBOSS package as well as links to WEB-Services can be found at <http://emboss.sourceforge.net>. A preferred, non-limiting example of parameters to be used for aligning two amino acid sequences using the needle program are the default 10 parameters, including the EBLOSUM62 scoring matrix, a gap opening penalty of 10 and a gap extension penalty of 0.5. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the needle program in the EMBOSS software package (EMBOSS: The European Molecular Biology Open Software Suite, Rice,P., Longden,!., and Bleasby,A, Trends in Genetics 16(6), 276-277, 2000), using 15 the EDNAFULL scoring matrix and a gap opening penalty of 16, 14, 12, 10, 8, 6, or 4 and a gap extension penalty of 0.5,1 , 2, 3, 4, 5, or 6. A preferred, non-limiting example of parameters to be used in conjunction for aligning two amino acid sequences using the needle program are the default parameters, including the EDNAFULL scoring matrix, a gap opening penalty of 10 and a gap extension penalty of 0.5. The nucleic acid and protein 20 sequences of the present invention can further be used as a " query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the BLAST series of programs (version 2.2) of Altschul et al. (Altschul 1990, J. Mol. Biol. 215:403-10). BLAST using acyltransferase nucleic acid sequences of the invention as query sequence can be 25 performed with the BLASTn, BLASTx or the tBLASTx program using default parameters to obtain either nucleotide sequences (BLASTn, tBLASTx) or amino acid sequences (BLASTx) homologous to acyltransferase sequences of the invention. BLAST using acyltransferase protein sequences of the invention as query sequence can be performed with the BLASTp or the tBLASTn program using default parameters to obtain either amino acid sequences 30 (BLASTp) or nucleic acid sequences (tBLASTn) homologous to acyltransferase sequences of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST using default parameters can be utilized as described in Altschul et al. (Altschul 1997, Nucleic Acids Res. 25(17):3389-3402).

35 Table 1: Relation of sequence types of querry and hit sequences for various BLASt programs

Input query sequence	Converted Query	Algorithm	Converted Hit	Actual Database
DNA		BLASTn		DNA
PRT		BLASTp		PRT
DNA	PRT	BLASTx		PRT

PRT		tBLASTn	PRT	DNA
DNA	PRT	tBLASTx	PRT	DNA

A polynucleotide comprising a fragment of any of the aforementioned nucleic acid sequences is also encompassed as a polynucleotide of the present invention. The fragment shall encode a polypeptide which still has desaturase and elongase activity as specified above. Accordingly, the polypeptide may comprise or consist of the domains of the polypeptide of the present invention conferring the said biological activity. A fragment as meant herein, preferably, comprises at least 50, at least 100, at least 250 or at least 500 consecutive nucleotides of any one of the aforementioned nucleic acid sequences or encodes an amino acid sequence comprising at least 20, at least 30, at least 50, at least 80, at least 100 or at least 150 consecutive amino acids of any one of the aforementioned amino acid sequences.

The variant polynucleotides or fragments referred to above, preferably, encode polypeptides retaining desaturase or elongase activity to a significant extent, preferably, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the desaturase and elongase activity exhibited by any of the polypeptide shown in any one of SEQ ID NOs: 2, 4, 6, 8 or 10. The activity may be tested as described in the accompanying Examples.

The polynucleotides of the present invention either essentially consist of the aforementioned nucleic acid sequences or comprise the aforementioned nucleic acid sequences. Thus, they may contain further nucleic acid sequences as well. Preferably, the polynucleotide of the present invention may comprise in addition to an open reading frame further untranslated sequence at the 3' and at the 5' terminus of the coding gene region: at least 500, preferably 200, more preferably 100 nucleotides of the sequence upstream of the 5' terminus of the coding region and at least 100, preferably 50, more preferably 20 nucleotides of the sequence downstream of the 3' terminus of the coding gene region. Furthermore, the polynucleotides of the present invention may encode fusion proteins wherein one partner of the fusion protein is a polypeptide being encoded by a nucleic acid sequence recited above. Such fusion proteins may comprise as additional part other enzymes of the fatty acid or PUFA biosynthesis pathways, polypeptides for monitoring expression (e.g., green, yellow, blue or red fluorescent proteins, alkaline phosphatase and the like) or so called "tags" which may serve as a detectable marker or as an auxiliary measure for purification purposes. Tags for the different purposes are well known in the art and comprise FLAG-tags, 6-histidine-tags, MYC-tags and the like.

The polynucleotide of the present invention shall be provided, preferably, either as an isolated polynucleotide (i.e. purified or at least isolated from its natural context such as its natural gene locus) or in genetically modified or exogenously (i.e. artificially) manipulated form. An isolated polynucleotide can, for example, comprise less than approximately 5 kb, 4

kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in the genomic DNA of the cell from which the nucleic acid is derived. The polynucleotide, preferably, is provided in the form of double or single stranded molecule. It will be understood that the present invention by referring to any of the

5 aforementioned polynucleotides of the invention also refers to complementary or reverse complementary strands of the specific sequences or variants thereof referred to before. The polynucleotide encompasses DNA, including cDNA and genomic DNA, or RNA polynucleotides.

10 However, the present invention also pertains to polynucleotide variants which are derived from the polynucleotides of the present invention and are capable of interfering with the transcription or translation of the polynucleotides of the present invention. Such variant polynucleotides include anti-sense nucleic acids, ribozymes, siRNA molecules, morpholino nucleic acids (phosphorodiamidate morpholino oligos), triple-helix forming oligonucleotides, 15 inhibitory oligonucleotides, or micro RNA molecules all of which shall specifically recognize the polynucleotide of the invention due to the presence of complementary or substantially complementary sequences. These techniques are well known to the skilled artisan. Suitable variant polynucleotides of the aforementioned kind can be readily designed based on the structure of the polynucleotides of this invention.

20 Moreover, comprised are also chemically modified polynucleotides including naturally occurring modified polynucleotides such as glycosylated or methylated polynucleotides or artificial modified ones such as biotinylated polynucleotides.

25 In the studies underlying the present invention, advantageously, polynucleotides where identified encoding desaturase and elongases from *Emiliana huxleyi*. In particular, the *Emiliana huxleyi* desaturases $\Delta 4\text{Des(Eh)}$, $\Delta 8\text{Des(Eh)}$ and $\Delta 5\text{Des(Eh)}$ and elongases $\Delta 9\text{Elo(Eh)}$ and $\Delta 5\text{Elo(Eh)}$ have been identified. Each of the desaturases are capable of introducing a double bond into fatty acids. For example, the expression of the $\Delta 8\text{Des(Eh)}$ 30 leads to introduction of a double bond at position eight into C20:2n-6 fatty acid. The polynucleotides of the present invention are particularly suitable for the recombinant manufacture of LCPUFAs and, in particular, ARA, EPA and/or DHA.

In a preferred embodiment of the polynucleotide of the present invention, said 35 polynucleotide further comprises an expression control sequence operatively linked to the said nucleic acid sequence.

The term "expression control sequence" as used herein refers to a nucleic acid sequence which is capable of governing, i.e. initiating and controlling, transcription of a 40 nucleic acid sequence of interest, in the present case the nucleic sequences recited above. Such a sequence usually comprises or consists of a promoter or a combination of a promoter and enhancer sequences. Expression of a polynucleotide comprises transcription

of the nucleic acid molecule, preferably, into a translatable mRNA. Additional regulatory elements may include transcriptional as well as translational enhancers. The following promoters and expression control sequences may be, preferably, used in an expression vector according to the present invention. The cos, tac, trp, tet, trp-tet, lpp, lac, lpp-lac, laclq, T7, T5, T3, gal, trc, ara, SP6, λ -PR or λ -PL promoters are, preferably, used in Gram-negative bacteria. For Gram-positive bacteria, promoters amy and SPO2 may be used. From yeast or fungal promoters ADC1, AOX1 r, GAL1, MFa, AC, P-60, CYC1, GAPDH, TEF, rp28, ADH are, preferably, used. For animal cell or organism expression, the promoters CMV-, SV40-, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer are preferably used. From plants the promoters CaMV/35S (Franck 1980, Cell 21: 285-294], PRP1 (Ward 1993, Plant. Mol. Biol. 22), SSU, OCS, lib4, usp, STLS1, B33, nos or the ubiquitin or phaseolin promoter. Also preferred in this context are inducible promoters, such as the promoters described in EP 0 388 186 A 1 (i.e. a benzylsulfonamide-inducible promoter), Gatz 1992, Plant J. 2:397-404 (i.e. a tetracyclin-inducible promoter), EP 0 335 528 A 1 (i.e. a abscisic-acid-inducible promoter) or WO 93/21334 (i.e. a ethanol- or cyclohexenol-inducible promoter). Further suitable plant promoters are the promoter of cytosolic FBPase or the ST-LSI promoter from potato (Stockhaus 1989, EMBO J. 8, 2445), the phosphoribosyl-pyrophosphate amidotransferase promoter from Glycine max (Genbank accession No. U87999) or the node-specific promoter described in EP 0 249 676 A 1.

Particularly preferred are promoters which enable the expression in tissues which are involved in the biosynthesis of fatty acids. Also particularly preferred are seed-specific promoters such as the USP promoter in accordance with the practice, but also other promoters such as the LeB4, DC3, phaseolin or napin promoters. Further especially preferred promoters are seed-specific promoters which can be used for monocotyledonous or dicotyledonous plants and which are described in US 5,608,152 (napin promoter from oilseed rape), WO 98/45461 (oleosin promoter from Arobidopsis, US 5,504,200 (phaseolin promoter from Phaseolus vulgaris), WO 91/13980 (Bce4 promoter from Brassica), by Baeumlein et al., Plant J., 2, 2, 1992:233-239 (LeB4 promoter from a legume), these promoters being suitable for dicots. The following promoters are suitable for monocots: Ipt-2 or Ipt-1 promoter from barley (WO 95/1 5389 and WO 95/23230), hordein promoter from barley and other promoters which are suitable and which are described in WO 99/16890. In principle, it is possible to use all natural promoters together with their regulatory sequences, such as those mentioned above, for the novel process. Likewise, it is possible and advantageous to use synthetic promoters, either additionally or alone, especially when they mediate a seed-specific expression, such as, for example, as described in WO 99/16890. In a particular embodiment, seed-specific promoters are utilized to enhance the production of the desired PUFA or LCPUFA.

The term "operatively linked" as used herein means that the expression control sequence and the nucleic acid of interest are linked so that the expression of the said nucleic acid of interest can be governed by the said expression control sequence, i.e. the expression control sequence shall be functionally linked to the said nucleic acid sequence

to be expressed. Accordingly, the expression control sequence and, the nucleic acid sequence to be expressed may be physically linked to each other, e.g., by inserting the expression control sequence at the 5' end of the nucleic acid sequence to be expressed. Alternatively, the expression control sequence and the nucleic acid to be expressed may be merely in physical proximity so that the expression control sequence is capable of governing the expression of at least one nucleic acid sequence of interest. The expression control sequence and the nucleic acid to be expressed are, preferably, separated by not more than 500 bp, 300 bp, 100 bp, 80 bp, 60 bp, 40 bp, 20 bp, 10 bp or 5 bp.

- 5 10 In a further preferred embodiment of the polynucleotide of the present invention, said polynucleotide further comprises a terminator sequence operatively linked to the nucleic acid sequence.

The term " terminator" as used herein refers to a nucleic acid sequence which is capable 15 of terminating transcription. These sequences will cause dissociation of the transcription machinery from the nucleic acid sequence to be transcribed. Preferably, the terminator shall be active in plants and, in particular, in plant seeds. Suitable terminators are known in the art and, preferably, include polyadenylation signals such as the SV40-poly-A site or the tk-poly-A site or one of the plant specific signals indicated in Loke et al. (Loke 2005, Plant 20 Physiol 138, pp. 1457-1468), downstream of the nucleic acid sequence to be expressed.

The present invention also relates to a vector comprising the polynucleotide of the present invention.

- 25 The term " vector" , preferably, encompasses phage, plasmid, viral vectors as well as artificial chromosomes, such as bacterial or yeast artificial chromosomes. Moreover, the term also relates to targeting constructs which allow for random or site- directed integration of the targeting construct into genomic DNA. Such target constructs, preferably, comprise DNA of sufficient length for either homologous or heterologous recombination as described in 30 detail below. The vector encompassing the polynucleotide of the present invention, preferably, further comprises selectable markers for propagation and/or selection in a host. The vector may be incorporated into a host cell by various techniques well known in the art. If introduced into a host cell, the vector may reside in the cytoplasm or may be incorporated into the genome. In the latter case, it is to be understood that the vector may further 35 comprise nucleic acid sequences which allow for homologous recombination or heterologous insertion. Vectors can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. The terms " transformation" and " transfection" , conjugation and transduction, as used in the present context, are intended to comprise a multiplicity of prior-art processes for introducing foreign nucleic acid (for 40 example DNA) into a host cell, including calcium phosphate, rubidium chloride or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, natural competence, carbon-based clusters, chemically mediated transfer, electroporation or

particle bombardment. Suitable methods for the transformation or transfection of host cells, including plant cells, can be found in Sambrook et al. (*Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) and other laboratory manuals, such as *Methods in Molecular*

5 *Biology*, 1995, Vol. 44, *Agrobacterium protocols*, Ed: Gartland and Davey, Humana Press, Totowa, New Jersey. Alternatively, a plasmid vector may be introduced by heat shock or electroporation techniques. Should the vector be a virus, it may be packaged in vitro using an appropriate packaging cell line prior to application to host cells.

10 Preferably, the vector referred to herein is suitable as a cloning vector, i.e. replicable in microbial systems. Such vectors ensure efficient cloning in bacteria and, preferably, yeasts or fungi and make possible the stable transformation of plants. Those which must be mentioned are, in particular, various binary and co-integrated vector systems which are suitable for the T-DNA-mediated transformation. Such vector systems are, as a rule,

15 characterized in that they contain at least the vir genes, which are required for the *Agrobacterium*-mediated transformation, and the sequences which delimit the T-DNA (T-DNA border). These vector systems, preferably, also comprise further cis-regulatory regions such as promoters and terminators and/or selection markers with which suitable transformed host cells or organisms can be identified. While co-integrated vector systems

20 have vir genes and T-DNA sequences arranged on the same vector, binary systems are based on at least two vectors, one of which bears vir genes, but no T-DNA, while a second one bears T-DNA, but no vir gene. As a consequence, the last-mentioned vectors are relatively small, easy to manipulate and can be replicated both in *E. coli* and in *Agrobacterium*. These binary vectors include vectors from the pBIB-HYG, pPZP, pBecks,

25 pGreen series. Preferably used in accordance with the invention are Bin19, pBI101, pBinAR, pGPTV and pCAMBIA. An overview of binary vectors and their use can be found in Hellens et al, *Trends in Plant Science* (2000) 5, 446- 451 . Furthermore, by using appropriate cloning vectors, the polynucleotides can be introduced into host cells or organisms such as plants or animals and, thus, be used in the transformation of plants,

30 such as those which are published, and cited, in: *Plant Molecular Biology and Biotechnology* (CRC Press, Boca Raton, Florida), chapter 6/7, pp. 71-119 (1993); F.F. White, *Vectors for Gene Transfer in Higher Plants*; in: *Transgenic Plants*, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, 15-38; B. Jenes et al., *Techniques for Gene Transfer*, in: *Transgenic Plants*, vol. 1, Engineering and

35 Utilization, Ed.: Kung and R. Wu, Academic Press (1993), 128-143; Potrykus 1991, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42, 205-225.

More preferably, the vector of the present invention is an expression vector. In such an expression vector, i.e. a vector which comprises the polynucleotide of the invention having 40 the nucleic acid sequence operatively linked to an expression control sequence (also called " expression cassette") allowing expression in prokaryotic or eukaryotic cells or isolated fractions thereof. Suitable expression vectors are known in the art such as Okayama-Berg

- cDNA expression vector pcDV1 (Pharmacia), pCDM δ , pRc/CMV, pcDNAI , pcDNA3 (Invitrogen) or pSPORTI (GIBCO BRL). Further examples of typical fusion expression vectors are pGEX (Pharmacia Biotech Inc; Smith 1988, Gene 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ), where glutathione S-transferase (GST), maltose E-binding protein and protein A, respectively, are fused with the recombinant target protein. Examples of suitable inducible nonfusion E. coli expression vectors are, inter alia, pTrc (Amann 1988, Gene 69:301-315) and pET 11d (Studier 1990, Methods in Enzymology 185, 60-89). The target gene expression of the pTrc vector is based on the transcription from a hybrid trp-lac fusion promoter by host RNA polymerase.
- The target gene expression from the pET 11d vector is based on the transcription of a T7-gn10-lac fusion promoter, which is mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is provided by the host strains BL21 (DE3) or HMS174 (DE3) from a resident λ -prophage which harbors a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter. The skilled worker is familiar with other vectors which are suitable in prokaryotic organisms; these vectors are, for example, in E. coli, pLG338, pACYC184, the pBR series such as pBR322, the pUC series such as pUC18 or pUC19, the M113mp series, pKC30, pRep4, pHs1 , pHs2, pPLc236, pMBL24, pLG200, pUR290, pIN-1111 13-B1 , λ gt1 1 or pBdCl, in Streptomyces plJ101 , plJ364, plJ702 or plJ361 , in Bacillus pUB1 10, pC194 or pBD214, in Corynebacterium pSA77 or pAJ667. Examples of vectors for expression in the yeast S. cerevisiae comprise pYep Sec1 (Baldari 1987, Embo J. 6:229-234), pMFa (Kurjan 1982, Cell 30:933-943), pJRY88 (Schultz 1987, Gene 54:1 13-123) and pYES2 (Invitrogen Corporation, San Diego, CA). Vectors and processes for the construction of vectors which are suitable for use in other fungi, such as the filamentous fungi, comprise those which are described in detail in: van den Hondel, C.A.M.J.J., & Punt, P.J. (1991) " Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of fungi, J.F. Peberdy et al., Ed., pp. 1-28, Cambridge University Press: Cambridge, or in: More Gene Manipulations in Fungi (J.W. Bennett & L.L. Lasure, Ed., pp. 396-428: Academic Press: San Diego). Further suitable yeast vectors are, for example, pAG-1 , YEp6, YEp13 or pEMBLYe23. As an alternative, the polynucleotides of the present invention can be also expressed in insect cells using baculovirus expression vectors. Baculovirus vectors which are available for the expression of proteins in cultured insect cells (for example Sf9 cells) comprise the pAc series (Smith 1983, Mol. Cell Biol. 3:2156-2165) and the pVL series (Lucklow 1989, Virology 170:31-39).
- The polynucleotide of the present invention can be expressed in single-cell plant cells (such as algae), see Falciatore 1999, Marine Biotechnology 1 (3):239-251 and the references cited therein, and plant cells from higher plants (for example Spermatophytes, such as arable crops) by using plant expression vectors. Examples of plant expression vectors comprise those which are described in detail in: Becker 1992, Plant Mol. Biol. 20:1 195-1197; Bevan 1984, Nucl. Acids Res. 12:871 1-8721 ; Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, p. 15-38. A plant expression cassette, preferably, comprises

regulatory sequences which are capable of controlling the gene expression in plant cells and which are functionally linked so that each sequence can fulfill its function, such as transcriptional termination, for example polyadenylation signals. Preferred polyadenylation signals are those which are derived from *Agrobacterium tumefaciens* T-DNA, such as the 5 gene 3 of the Ti plasmid pTiACH5, which is known as octopine synthase (Gielen 1984, EMBO J. 3, 835) or functional equivalents of these, but all other terminators which are functionally active in plants are also suitable. Since plant gene expression is very often not limited to transcriptional levels, a plant expression cassette preferably comprises other functionally linked sequences such as translation enhancers, for example the overdrive 10 sequence, which comprises the 5' -untranslated tobacco mosaic virus leader sequence, which increases the protein/RNA ratio (GalNe 1987, Nucl. Acids Research 15:8693-871 1). As described above, plant gene expression must be functionally linked to a suitable promoter which performs the expression of the gene in a timely, cell-specific or tissue-specific manner. Promoters which can be used are constitutive promoters (Benfey 1989, 15 EMBO J. 8:2195-2202) such as those which are derived from plant viruses such as 35S CAMV (Franck 1980, Cell 21:285-294), 19S CaMV (see US 5,352,605 and WO 84/02913) or plant promoters such as the promoter of the Rubisco small subunit, which is described in US 4,962,028. Other preferred sequences for the use in functional linkage in plant gene expression cassettes are targeting sequences which are required for targeting the gene 20 product into its relevant cell compartment (for a review, see Kermode 1996, Crit. Rev. Plant Sci. 15, 4: 285-423 and references cited therein), for example into the vacuole, the nucleus, all types of plastids, such as amyloplasts, chloroplasts, chromoplasts, the extracellular space, the mitochondria, the endoplasmic reticulum, oil bodies, peroxisomes and other compartments of plant cells. As described above, plant gene expression can also be 25 facilitated via a chemically inducible promoter (for a review, see Gatz 1997, Annu. Rev. Plant Physiol. Plant Mol. Biol., 48:89-108). Chemically inducible promoters are particularly suitable if it is desired that genes are expressed in a time-specific manner. Examples of such promoters are a salicylic-acid-inducible promoter (WO 95/19443), a tetracyclin-inducible promoter (Gatz 1992, Plant J. 2, 397-404) and an ethanol-inducible promoter. 30 Promoters which respond to biotic or abiotic stress conditions are also suitable promoters, for example the pathogen-induced PRP1-gene promoter (Ward 1993, Plant Mol. Biol. 22:361-366), the heat-inducible hspδO promoter from tomato (US 5,187,267), the cold-inducible alpha-amylase promoter from potato (WO 96/12814) or the wound-inducible pin1 promoter (EP 0 375 091 A). The promoters which are especially preferred are those which 35 bring about the expression of genes in tissues and organs in which fatty acid, lipid and oil biosynthesis takes place, in seed cells such as the cells of endosperm and of the developing embryo. Suitable promoters are the napin gene promoter from oilseed rape (US 5,608,152), the USP promoter from *Vicia faba* (Baeumlein 1991, Mol. Gen. Genet. 225 (3):459-67), the oleosin promoter from *Arabidopsis* (WO 98/45461), the phaseolin promoter 40 from *Phaseolus vulgaris* (US 5,504,200), the Bce4 promoter from *Brassica* (WO 91/13980) or the legumin B4 promoter (LeB4; Baeumlein 1992, Plant Journal, 2 (2):233-9), and promoters which bring about the seed-specific expression in monocotyledonous plants such

as maize, barley, wheat, rye, rice and the like. Suitable promoters to be taken into consideration are the Ipt2 or Ipt1 gene promoter from barley (WO 95/15389 and WO 95/23230) or those which are described in WO 99/16890 (promoters from the barley hordein gene, the rice glutelin gene, the rice oryzin gene, the rice prolamin gene, the wheat 5 gliadin gene, wheat glutelin gene, the maize zein gene, the oat glutelin gene, the sorghum kasirin gene, the rye secalin gene). Likewise, especially suitable are promoters which bring about the plastid-specific expression since plastids are the compartment in which the precursors and some end products of lipid biosynthesis are synthesized. Suitable promoters such as the viral RNA-polymerase promoter, are described in WO 95/16783 and WO 10 97/06250, and the clpP promoter from Arabidopsis, described in WO 99/46394.

The abovementioned vectors are only a small overview of vectors to be used in accordance with the present invention. Further vectors are known to the skilled worker and are described, for example, in: Cloning Vectors (Ed., Pouwels, P.H., et al., Elsevier, 15 Amsterdam-New York-Oxford, 1985, ISBN 0 444 90401 8). For further suitable expression systems for prokaryotic and eukaryotic cells see the chapters 16 and 17 of Sambrook, loc cit.

It follows from the above that, preferably, said vector is an expression vector. More 20 preferably, the said polynucleotide of the present invention is under the control of a seed-specific promoter in the vector of the present invention. A preferred seed-specific promoter as meant herein is selected from the group consisting of Conlinin 1, Conlinin 2, napin, LuFad3, USP, LeB4, Arc, Fae, ACP, LuPXR, and SBP. For details, see, e.g., US 2003-0159174.

25 Moreover, the present invention relates to a host cell comprising the polynucleotide or the vector of the present invention.

Preferably, said host cell is a plant cell and, more preferably, a plant cell obtained from an 30 oilseed crop. More preferably, said oilseed crop is selected from the group consisting of flax (Linum sp.), rapeseed (Brassica sp.), soybean (Glycine sp.), sunflower (Helianthus sp.), cotton (Gossypium sp.), corn (Zea mays), olive (Olea sp.), safflower (Carthamus sp.), cocoa (Theobroma cacao), peanut (Arachis sp.), hemp, camelina, crambe, oil palm, coconuts, groundnuts, sesame seed, castor bean, lesquerella, tallow tree, sheanuts, tungnuts, kapok 35 fruit, poppy seed, jojoba seeds and perilla.

Also preferably, said host cell is a microorganism. More preferably, said microorganism is a bacterium, a fungus or algae. More preferably, it is selected from the group consisting of Candida, Cryptococcus, Lipomyces, Rhodosporidium, Yarrowia and Schizochytrium.

40 Moreover, a host cell according to the present invention may also be an animal cell. Preferably, said animal host cell is a host cell of a fish or a cell line obtained therefrom.

More preferably, the fish host cell is from herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna.

Generally, the controlling steps in the production of LCPUFAs, i.e., the long chain

5 unsaturated fatty acid biosynthetic pathway, are catalyzed by membrane-associated fatty acid desaturases and elongases. Plants and most other eukaryotic organisms have specialized desaturase and elongase systems for the introduction of double bonds and the extension of fatty acids beyond C18 atoms. The elongase reactions have several important features in common with the fatty acid synthase complex (FAS). However, the elongase
10 complex is different from the FAS complex as the complex is localized in the cytosol and membrane bound, ACP is not involved and the elongase 3-keto-acyl-CoA-synthase catalyzes the condensation of malonyl-CoA with an acyl primer. The elongase complex consists of four components with different catalytic functions, the keto-acyl-synthase (condensation reaction of malonyl-CoA to acyl-CoA, creation of a 2 C atom longer keto-
15 acyl-CoA fatty acid), the keto-acyl-reductase (reduction of the 3-keto group to a 3-hydroxy-group), the dehydratase (dehydration results in a 3-enoyl-acyl-CoA fatty acid) and the enoyl-CoA-reductase (reduction of the double bond at position 3, release from the complex). For the production of LCPUFAs including ARA, EPA and/or DHA the elongation reactions, beside the desaturation reactions, are essential. Higher plants do not have the
20 necessary enzyme set to produce LCPUFAs (4 or more double bonds, 20 or more C atoms). Therefore the catalytic activities have to be conferred to the plants or plant cells. The polynucleotides of the present invention catalyze the desaturation and elongation activities necessary for the formation of ARA, EPA and/or DHA. By delivering the novel desaturases and elongases increased levels of PUFAs and LCPUFAs are produced.

25 However, person skilled in the art knows that dependent on the host cell, further, enzymatic activities may be conferred to the host cells, e.g., by recombinant technologies. Accordingly, the present invention, preferably, envisages a host cell which in addition to the polynucleotide of the present invention comprises polynucleotides encoding such desaturases and/or elongases as required depending on the selected host cell. Preferred
30 desaturases and/or elongases which shall be present in the host cell are at least one enzyme selected from the group consisting of: Δ -4-desaturase, Δ -5-desaturase, Δ -5-elongase, Δ -6-desaturase, Δ 12-desaturase, Δ 15-desaturase, ω 3-desaturase and Δ -6-elongase. Especially preferred are the bifunctional d12d15-Desaturases d12d15Des(Ac)
35 from Acanthamoeba castellanii (WO2007042510), d12d15Des(Cp) from Claviceps purpurea (WO2008006202) and d12d15Des(Lg1) from Lottia gigantea (WO2009016202), the d12-Desaturases d12Des(Co) from Calendula officinalis (WO200185968), d12Des(Lb) from Laccaria bicolor (WO2009016202), d12Des(Mb) from Monosiga brevicollis (WO2009016202), d12Des(Mg) from Mycosphaerella graminicola (WO2009016202),
40 d12Des(Nh) from Nectria haematococca (WO2009016202), d12Des(OI) from Ostreococcus lucimarinus (WO2008040787), d12Des(Pb) from Phycomyces blakesleeanus (WO2009016202), d12Des(Ps) from Phytophthora sojae (WO2006100241) and

d12Des(Tp) from *Thalassiosira pseudonana* (WO2006069710), the d15-Desaturases d15Des(Hr) from *Helobdella robusta* (WO20090 16202), d15Des(Mc) from *Microcoleus chthonoplastes* (WO2009016202), d15Des(Mf) from *Mycosphaerella fijiensis* (WO20090 16202), d15Des(Mg) from *Mycosphaerella graminicola* (WO20090 16202) and

5 d15Des(Nh)2 from *Nectria haematococca* (WO2009016202), the d4-Desaturases d4Des(Eg) from *Euglena gracilis* (WO2004090123), d4Des(Tc) from *Thraustochytrium sp.* (WO2002026946) and d4Des(Tp) from *Thalassiosira pseudonana* (WO2006069710), the d5-Desaturases d5Des(OI)2 from *Ostreococcus lucimarinus* (WO2008040787), d5Des(Pp) from *Physcomitrella patens* (WO2004057001), d5Des(Pt) from *Phaeodactylum tricornutum*

10 (WO2002057465), d5Des(Tc) from *Thraustochytrium sp.* (WO2002026946), d5Des(Tp) from *Thalassiosira pseudonana* (WO2006069710) and the d6-Desaturases d6Des(Cp) from *Ceratodon purpureus* (WO2000075341), d6Des(OI) from *Ostreococcus lucimarinus* (WO2008040787), d6Des(Ot) from *Ostreococcus tauri* (WO2006069710), d6Des(Pf) from *Primula farinosa* (WO2003072784), d6Des(Pir)_BO from *Pythium irregularare* (WO2002026946), d6Des(Pir) from *Pythium irregularare* (WO2002026946), d6Des(Plu) from *Primula luteola* (WO2003072784), d6Des(Pp) from *Physcomitrella patens* (WO200 102591), d6Des(Pt) from *Phaeodactylum tricornutum* (WO2002057465), d6Des(Pv) from *Primula vialii* (WO2003072784) and d6Des(Tp) from *Thalassiosira pseudonana* (WO2006069710), the d8-Desaturases d8Des(Ac) from *Acanthamoeba castellanii* (EP1 790731), d δ Des(Eg)

15 20 from *Euglena gracilis* (WO20034439) and d δ Des(Pm) from *Perkinsus marinus* (WO2007093776), the o3-Desaturases o3Des(Pi) from *Phytophthora infestans* (WO2005083053), o3Des(Pir) from *Pythium irregularare* (WO2008022963), o3Des(Pir)2 from *Pythium irregularare* (WO2008022963) and o3Des(Ps) from *Phytophthora sojae* (WO20061 00241), the bifunctional d5d6-elongases d5d6Elo(O m)2 from *Oncorhynchus mykiss* (WO2005012316), d5d6Elo(Ta) from *Thraustochytrium aureum* (WO2005012316) and d5d6Elo(Tc) from *Thraustochytrium sp.* (WO2005012316), the d5-elongases d5Elo(At) from *Arabidopsis thaliana* (WO2005012316), d5Elo(At)2 from *Arabidopsis thaliana* (WO2005012316), d5Elo(Ci) from *Ciona intestinalis* (WO2005012316), d5Elo(OI) from *Ostreococcus lucimarinus* (WO2008040787), d5Elo(Ot) from *Ostreococcus tauri* (WO2005012316), d5Elo(Tp) from *Thalassiosira pseudonana* (WO2005012316) and d5Elo(XI) from *Xenopus laevis* (WO2005012316), the d6-elongases d6Elo(OI) from *Ostreococcus lucimarinus* (WO2008040787), d6Elo(Ot) from *Ostreococcus tauri* (WO2005012316), d6Elo(Pi) from *Phytophthora infestans* (WO2003064638), d6Elo(Pir) from *Pythium irregularare* (WO2009016208), d6Elo(Pp) from *Physcomitrella patens* (WO200 10591 28), d6Elo(Ps) from *Phytophthora sojae* (WO20061 00241), d6Elo(Ps)2 from *Phytophthora sojae* (WO20061 00241), d6Elo(Ps)3 from *Phytophthora sojae* (WO20061 00241), d6Elo(Pt) from *Phaeodactylum tricornutum* (WO2005012316), d6Elo(Tc) from *Thraustochytrium sp.* (WO2005012316) and d6Elo(Tp) from *Thalassiosira pseudonana* (WO2005012316), the d9-elongases d9Elo(lg) from *Isochrysis galbana* (WO2002077213),

30 35 40 d9Elo(Pm) from *Perkinsus marinus* (WO2007093776) and d9Elo(Ro) from *Rhizopus oryzae* (WO2009016208). Particularly, if the manufuature of ARA is envisaged in higher plants, the enzymes recited in Table 3, below (i.e. aditinonally a d6-desaturase, d6-elongase, d5-

elongase, d5-desaturase, d12-desaturase, and d6-elongase) or enzymes having essentially the same activity may be combined in a host cell. If the manufacture of EPA is envisaged in higher plants, the enzymes recited in Table 4, below (i.e. aditinonally a d6-desaturase, d6-elongase, d5-desaturase, d12-desaturase, d6-elongase, omega 3-desaturase and d15-

- 5 desaturase), or enzymes having essentially the same activity may be combined in a host cell. If the manufacture of DHA is envisaged in higher plants, the enzymes recited in Table 5, below (i.e. aditinonally a d6-desaturase, d6-elongase, d5-desaturase, d12-desaturase, d6-elongase, omega 3-desaturase, d15-desaturase, d5-elongase, and d4-desaturase), or enzymes having essentially the same activity may be combined in a host cell.

10

The present invention also relates to a cell, preferably a host cell as specified above or a cell of a non-human organism specified elsewhere herein, said cell comprising a polynucleotide which is obtained from the polynucleotide of the present invention by a point mutation, a truncation, an inversion, a deletion, an addition, a substitution and homologous 15 recombination. How to carry out such modifications to a polynucleotide is well known to the skilled artisan and has been described elsewhere in this specification in detail.

The present invention furthermore pertains to a method for the manufacture of a polypeptide encoded by a polynucleotide of any the present invention comprising
20 a) cultivating the host cell of the invention under conditions which allow for the production of the said polypeptide; and
b) obtaining the polypeptide from the host cell of step a).

Suitable conditions which allow for expression of the polynucleotide of the invention
25 comprised by the host cell depend on the host cell as well as the expression control sequence used for governing expression of the said polynucleotide. These conditions and how to select them are very well known to those skilled in the art. The expressed polypeptide may be obtained, for example, by all conventional purification techniques including affinity chromatography, size exclusion chromatography, high pressure liquid
30 chromatography (HPLC) and precipitation techniques including antibody precipitation. It is to be understood that the method may - although preferred -not necessarily yield an essentially pure preparation of the polypeptide. It is to be understood that depending on the host cell which is used for the aforementioned method, the polypeptides produced thereby may become posttranslationally modified or processed otherwise.

35

The present invention also encompasses a polypeptide encoded by the polynucleotide of of the present invention or which is obtainable by the aforementioned method.

The term " polypeptide" as used herein encompasses essentially purified polypeptides or
40 polypeptide preparations comprising other proteins in addition. Further, the term also relates to the fusion proteins or polypeptide fragments being at least partially encoded by the polynucleotide of the present invention referred to above. Moreover, it includes

chemically modified polypeptides. Such modifications may be artificial modifications or naturally occurring modifications such as phosphorylation, glycosylation, myristylation and the like (Review in Mann 2003, Nat. Biotechnol. 21, 255- 261 , review with focus on plants in Huber 2004, Curr. Opin. Plant Biol. 7, 318-322). Currently, more than 300

5 posttranslational modifications are known (see full ABFRC Delta mass list at <http://www.abrf.org/index.cfm/dm.home>). The polypeptides of the present invention shall exhibit the desaturase or elongase activity referred to above.

Encompassed by the present invention is, furthermore, an antibody or fragments thereof

10 which specifically recognizes the polypeptide of the invention.

Antibodies against the polypeptides of the invention can be prepared by well known methods using a purified polypeptide according to the invention or a suitable fragment derived therefrom as an antigen. A fragment which is suitable as an antigen may be

15 identified by antigenicity determining algorithms well known in the art. Such fragments may be obtained either from the polypeptide of the invention by proteolytic digestion or may be a synthetic peptide. Preferably, the antibody of the present invention is a monoclonal antibody, a polyclonal antibody, a single chain antibody, a chimerized antibody or a fragment of any of these antibodies, such as Fab, Fv or scFv fragments etc.. Also 20 comprised as antibodies by the present invention are bispecific antibodies, synthetic antibodies or chemically modified derivatives of any of the aforementioned antibodies. The antibody of the present invention shall specifically bind (i.e. does significantly not cross react with other polypeptides or peptides) to the polypeptide of the invention. Specific binding can be tested by various well known techniques. Antibodies or fragments thereof

25 can be obtained by using methods which are described, e.g., in Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1988. Monoclonal antibodies can be prepared by the techniques originally described in Köhler 1975, Nature 256, 495, and Galfre 1981 , Meth. Enzymol. 73, 3, which comprise the fusion of mouse myeloma cells to spleen cells derived from immunized mammals. The antibodies can be 30 used, for example, for the immunoprecipitation, immunolocalization or purification (e.g., by affinity chromatography) of the polypeptides of the invention as well as for the monitoring of the presence of said variant polypeptides, for example, in recombinant organisms, and for the identification of proteins or compounds interacting with the proteins according to the invention.

35 Moreover, the present invention contemplates a non-human transgenic organism comprising the polynucleotide or the vector of the present invention.

Preferably, the non-human transgenic organism is a plant, plant part, or plant seed.

40 Preferred plants to be used for introducing the polynucleotide or the vector of the invention are plants which are capable of synthesizing fatty acids, such as all dicotyledonous or monocotyledonous plants, algae or mosses. It is to be understood that host cells derived

from a plant may also be used for producing a plant according to the present invention. Preferred plants are selected from the group of the plant families Adelotheciaceae, Anacardiaceae, Asteraceae, Apiaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Caricaceae, Cannabaceae, Convolvulaceae, Chenopodiaceae,

5 Cryptecodiniaceae, Cucurbitaceae, Ditrichaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Geraniaceae, Gramineae, Juglandaceae, Lauraceae, Leguminosae, Linaceae, Prasinophyceae or vegetable plants or ornamentals such as Tagetes. Examples which may be mentioned are the following plants selected from the group consisting of: Adelotheciaceae such as the genera Physcomitrella, such as the genus and species Physcomitrella patens, Anacardiaceae such as the genera Pistacia, Mangifera, Anacardium, for example the genus and species Pistacia vera [pistachio], Mangifer indica [mango] or Anacardium occidentale [cashew], Asteraceae, such as the genera Calendula, Carthamus, Centaurea, Cichorium, Cynara, Helianthus, Lactuca, Locusta, Tagetes, Valeriana, for example the genus and species Calendula officinalis [common marigold],

10 15 Carthamus tinctorius [safflower], Centaurea cyanus [cornflower], Cichorium intybus [chicory], Cynara scolymus [artichoke], Helianthus annus [sunflower], Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola L. ssp. sativa, Lactuca scariola L. var. integrata, Lactuca scariola L. var. integrifolia, Lactuca sativa subsp. romana, Locusta communis, Valeriana locusta [salad vegetables], Tagetes lucida, Tagetes erecta or Tagetes tenuifolia [african orfrench marigold], Apiaceae, such as the genus Daucus, for example the genus and species Daucus carota [carrot], Betulaceae, such as the genus Corylus, for example the genera and species Corylus avellana or Corylus colurna [hazelnut],

20 25 Boraginaceae, such as the genus Borago, for example the genus and species Borago officinalis [borage], Brassicaceae, such as the genera Brassica, Melanosinapis, Sinapis, Arabidopsis, for example the genera and species Brassica napus, Brassica rapa ssp. [oilseed rape], Sinapis arvensis Brassica juncea, Brassica juncea var. juncea, Brassica juncea var. crispifolia, Brassica juncea var. foliosa, Brassica nigra, Brassica sinapioides, Melanosinapis communis [mustard], Brassica oleracea [fodder beet] or Arabidopsis thaliana, Bromeliaceae, such as the genera Anana, Bromelia (pineapple), for example the genera and species Anana comosus, Ananas ananas or Bromelia comosa [pineapple],

30 35 Caricaceae, such as the genus Carica, such as the genus and species Carica papaya [pawpaw], Cannabaceae, such as the genus Cannabis, such as the genus and species Cannabis sativa [hemp], Convolvulaceae, such as the genera Ipomea, Convolvulus, for example the genera and species Ipomoea batatas, Ipomoea pandurata, Convolvulus batatas, Convolvulus tiliaceus, Ipomoea fastigiata, Ipomoea tiliacea, Ipomoea triloba or Convolvulus panduratus [sweet potato, batate], Chenopodiaceae, such as the genus Beta, such as the genera and species Beta vulgaris, Beta vulgaris var. altissima, Beta vulgaris var. Vulgaris, Beta maritima, Beta vulgaris var. perennis, Beta vulgaris var. conditiva or Beta vulgaris var. esculenta [sugarbeet], Cryptecodiniaceae, such as the genus

40 Cryptecodinium, for example the genus and species Cryptecodinium cohnii, Cucurbitaceae, such as the genus Cucurbita, for example the genera and species Cucurbita maxima, Cucurbita mixta, Cucurbita pepo or Cucurbita moschata

[pumpkin/squash], Cymbellaceae such as the genera *Amphora*, *Cymbella*, *Okedenia*, *Phaeodactylum*, *Reimeria*, for example the genus and species *Phaeodactylum tricornutum*, *Ditrichaceae* such as the genera *Ditrichaceae*, *Astomiopsis*, *Ceratodon*, *Chrysoblastella*, *Ditrichum*, *Distichium*, *Eccremidium*, *Lophidion*, *Philibertiella*, *Pleuridium*, *Saelania*,

5 *Trichodon*, *Skottsbergia*, for example the genera and species *Ceratodon antarcticus*, *Ceratodon columbiae*, *Ceratodon heterophyllus*, *Ceratodon purpureus*, *Ceratodon purpureus*, *Ceratodon purpureus* ssp. *convolutus*, *Ceratodon*, *purpureus* spp. *stenocarpus*, *Ceratodon* *purpureus* var. *rotundifolius*, *Ceratodon ratodon*, *Ceratodon stenocarpus*, *Chrysoblastella chilensis*, *Ditrichum ambiguum*, *Ditrichum brevisetum*, *Ditrichum*

10 *crispatissimum*, *Ditrichum difficile*, *Ditrichum falcifolium*, *Ditrichum flexicaule*, *Ditrichum giganteum*, *Ditrichum heteromallum*, *Ditrichum lineare*, *Ditrichum lineare*, *Ditrichum montanum*, *Ditrichum montanum*, *Ditrichum pallidum*, *Ditrichum punctulatum*, *Ditrichum pusillum*, *Ditrichum pusillum* var. *tortile*, *Ditrichum rhynchostegium*, *Ditrichum schimperi*, *Ditrichum tortile*, *Distichium capillaceum*, *Distichium hagenii*, *Distichium inclinatum*,

15 *Distichium macounii*, *Eccremidium floridanum*, *Eccremidium whiteleggei*, *Lophidion strictus*, *Pleuridium acuminatum*, *Pleuridium altemifolium*, *Pleuridium holdridgei*, *Pleuridium mexicanum*, *Pleuridium ravenelii*, *Pleuridium subulatum*, *Saelania glaucescens*, *Trichodon borealis*, *Trichodon cylindricus* or *Trichodon cylindricus* var. *oblongus*, *Elaeagnaceae* such as the genus *Elaeagnus*, for example the genus and species *Olea europaea* [olive],

20 *Ericaceae* such as the genus *Kalmia*, for example the genera and species *Kalmia latifolia*, *Kalmia angustifolia*, *Kalmia microphylla*, *Kalmia polifolia*, *Kalmia occidentalis*, *Cistus chamaerhodendros* or *Kalmia lucida* [mountain laurel], *Euphorbiaceae* such as the genera *Manihot*, *Janipha*, *Jatropha*, *Ricinus*, for example the genera and species *Manihot utilissima*, *Janipha manihot*, *Jatropha manihot*, *Manihot aipil*, *Manihot dulcis*, *Manihot manihot*, *Manihot melanobasis*, *Manihot esculenta* [*manihot*] or *Ricinus communis* [castor-oil plant], *Fabaceae* such as the genera *Pisum*, *Albizia*, *Cathormion*, *Feuillea*, *Inga*, *Pithecellobium*, *Acacia*, *Mimosa*, *Medicago*, *Glycine*, *Dolichos*, *Phaseolus*, *Soja*, for example the genera and species *Pisum sativum*, *Pisum arvense*, *Pisum humile* [pea], *Albizia berteriana*, *Albizia julibrissin*, *Albizia lebbeck*, *Acacia berteriana*, *Acacia littoralis*, *Albizia*

25 *berteriana*, *Albizzia berteriana*, *Cathormion berteriana*, *Feuillea berteriana*, *Inga fragrans*, *Pithecellobium berterianum*, *Pithecellobium fragrans*, *Pithecellobium berterianum*, *Pseudalbizzia berteriana*, *Acacia julibrissin*, *Acacia nemu*, *Albizia nemu*, *Feuillea julibrissin*, *Mimosa julibrissin*, *Mimosa speciosa*, *Sericanrda julibrissin*, *Acacia lebbeck*, *Acacia macrophylla*, *Albizia lebbek*, *Feuillea lebbeck*, *Mimosa lebbeck*, *Mimosa speciosa*

30 *[silk tree]*, *Medicago sativa*, *Medicago falcata*, *Medicago varia* [alfalfa], *Glycine max*, *Dolichos soja*, *Glycine gracilis*, *Glycine hispida*, *Phaseolus max*, *Soja hispida* or *Soja max* [soybean], *Funariaceae* such as the genera *Aphanorrhagma*, *Entosthodon*, *Funaria*, *Physcomitrella*, *Physcomitrium*, for example the genera and species *Aphanorrhagma serratum*, *Entosthodon attenuatus*, *Entosthodon bolanderi*, *Entosthodon bonplandii*,

35 *Entosthodon californicus*, *Entosthodon drummondii*, *Entosthodon jamesonii*, *Entosthodon leibergii*, *Entosthodon neoscoticus*, *Entosthodon rubrisetus*, *Entosthodon spathulifolius*, *Entosthodon tucsoni*, *Funaria americana*, *Funaria bolanderi*, *Funaria calcarea*, *Funaria*

californica, *Funaria calvescens*, *Funaria convoluta*, *Funaria flavicans*, *Funaria groutiana*, *Funaria hygrometrica*, *Funaria hygrometrica* var. *arctica*, *Funaria hygrometrica* var. *calvescens*, *Funaria hygrometrica* var. *convoluta*, *Funaria hygrometrica* var. *muralis*, *Funaria hygrometrica* var. *utahensis*, *Funaria microstoma*, *Funaria microstoma* var. 5 *obtusifolia*, *Funaria muhlenbergii*, *Funaria orcuttii*, *Funaria plano-convexa*, *Funaria polaris*, *Funaria ravenelii*, *Funaria rubriseta*, *Funaria serrata*, *Funaria sonorae*, *Funaria sublimbatus*, *Funaria tucsoni*, *Physcomitrella californica*, *Physcomitrella patens*, *Physcomitrella readeri*, *Physcomitrium australe*, *Physcomitrium californicum*, *Physcomitrium collenchymatum*, *Physcomitrium coloradense*, *Physcomitrium cupuliferum*, *Physcomitrium drummondii*, 10 *Physcomitrium eurystomum*, *Physcomitrium flexifolium*, *Physcomitrium hookeri*, *Physcomitrium hookeri* var. *serratum*, *Physcomitrium immersum*, *Physcomitrium kellermanii*, *Physcomitrium megalocarpum*, *Physcomitrium pyriforme*, *Physcomitrium pyriforme* var. *serratum*, *Physcomitrium rufipes*, *Physcomitrium sandbergii*, *Physcomitrium subsphaericum*, *Physcomitrium washingtoniense*, Geraniaceae, such as the genera 15 *Pelargonium*, *Cocos*, *Oleum*, for example the genera and species *Cocos nucifera*, *Pelargonium grossularioides* or *Oleum cocois* [coconut], Gramineae, such as the genus *Saccharum*, for example the genus and species *Saccharum officinarum*, Juglandaceae, such as the genera *Juglans*, WalNa, for example the genera and species *Juglans regia*, *Juglans ailanthifolia*, *Juglans sieboldiana*, *Juglans cinerea*, *Wallia cinerea*, *Juglans bixbyi*, 20 *Juglans californica*, *Juglans hindsii*, *Juglans intermedia*, *Juglans jamaicensis*, *Juglans major*, *Juglans microcarpa*, *Juglans nigra* or *Wallia nigra* [walnut], Lauraceae, such as the genera *Persea*, *Laurus*, for example the genera and species *Laurus nobilis* [bay], *Persea americana*, *Persea gratissima* or *Persea persea* [avocado], Leguminosae, such as the genus *Arachis*, for example the genus and species *Arachis hypogaea* [peanut], Linaceae, 25 such as the genera *Linum*, *Adenolinum*, for example the genera and species *Linum usitatissimum*, *Linum humile*, *Linum austriacum*, *Linum bienne*, *Linum angustifolium*, *Linum catharticum*, *Linum flavum*, *Linum grandiflorum*, *Adenolinum grandiflorum*, *Linum lewisii*, *Linum narbonense*, *Linum perenne*, *Linum perenne* var. *lewisii*, *Linum pratense* or *Linum trigynum* [linseed], Lythrarieae, such as the genus *Punica*, for example the genus and 30 species *Punica granatum* [pomegranate], Malvaceae, such as the genus *Gossypium*, for example the genera and species *Gossypium hirsutum*, *Gossypium arboreum*, *Gossypium barbadense*, *Gossypium herbaceum* or *Gossypium thurberi* [cotton], Marchantiaceae, such as the genus *Marchantia*, for example the genera and species *Marchantia berteroana*, *Marchantia foliacea*, *Marchantia macropora*, Musaceae, such as the genus *Musa*, for 35 example the genera and species *Musa nana*, *Musa acuminata*, *Musa paradisiaca*, *Musa spp.* [banana], Onagraceae, such as the genera *Camissonia*, *Oenothera*, for example the genera and species *Oenothera biennis* or *Camissonia brevipes* [evening primrose], Palmae, such as the genus *Elacis*, for example the genus and species *Elaeis guineensis* [oil palm], Papaveraceae, such as the genus *Papaver*, for example the genera and species *Papaver orientale*, *Papaver rhoeas*, *Papaver dubium* [poppy], Pedaliaceae, such as the genus *Sesamum*, for example the genus and species *Sesamum indicum* [sesame], Piperaceae, such as the genera *Piper*, *Artanthe*, *Peperomia*, *Steffensia*, for example the genera and 40

species *Piper aduncum*, *Piper amalago*, *Piper angustifolium*, *Piper auritum*, *Piper betel*, *Piper cubeba*, *Piper longum*, *Piper nigrum*, *Piper retrofractum*, *Artanthe adunca*, *Artanthe elongata*, *Peperomia elongata*, *Piper elongatum*, *Steffensia elongata* [cayenne pepper], Poaceae, such as the genera *Hordeum*, *Secale*, *Avena*, *Sorghum*, *Andropogon*, *Holcus*,

5 *Panicum*, *Oryza*, *Zea* (maize), *Triticum*, for example the genera and species *Hordeum vulgare*, *Hordeum jubatum*, *Hordeum murinum*, *Hordeum secalinum*, *Hordeum distichon*, *Hordeum aegiceras*, *Hordeum hexastichon*, *Hordeum hexastichum*, *Hordeum irregulare*, *Hordeum sativum*, *Hordeum secalinum* [barley], *Secale cereale* [rye], *Avena sativa*, *Avena fatua*, *Avena byzantina*, *Avena fatua* var. *sativa*, *Avena hybrida* [oats], *Sorghum bicolor*,

10 *Sorghum halepense*, *Sorghum saccharatum*, *Sorghum vulgare*, *Andropogon drummondii*, *Holcus bicolor*, *Holcus sorghum*, *Sorghum aethiopicum*, *Sorghum arundinaceum*, *Sorghum caffrorum*, *Sorghum cernuum*, *Sorghum dochna*, *Sorghum drummondii*, *Sorghum durra*, *Sorghum guineense*, *Sorghum lanceolatum*, *Sorghum nervosum*, *Sorghum saccharatum*, *Sorghum subglabrescens*, *Sorghum verticilliflorum*, *Sorghum vulgare*, *Holcus halepensis*,

15 *Sorghum miliaceum*, *Panicum militaceum* [millet], *Oryza sativa*, *Oryza latifolia* [rice], *Zea mays* [maize], *Triticum aestivum*, *Triticum durum*, *Triticum turgidum*, *Triticum hybemum*, *Triticum macha*, *Triticum sativum* or *Triticum vulgare* [wheat], Porphyridiaceae, such as the genera *Chroothece*, *Flintiella*, *Petrovanella*, *Porphyridium*, *Rhodella*, *Rhodosorus*, *Vanhoeffenia*, for example the genus and species *Porphyridium cruentum*, Proteaceae,

20 such as the genus *Macadamia*, for example the genus and species *Macadamia intergrifolia* [macadamia], Prasinophyceae such as the genera *Nephroselmis*, *Prasinococcus*, *Scherffelia*, *Tetraselmis*, *Mantoniella*, *Ostreococcus*, for example the genera and species *Nephroselmis olivacea*, *Prasinococcus capsulatus*, *Scherffelia dubia*, *Tetraselmis chui*, *Tetraselmis suecica*, *Mantoniella squamata*, *Ostreococcus tauri*, Rubiaceae such as the genus *Cofea*, for example the genera and species *Cofea* spp., *Coffea arabica*, *Coffea canephora* or *Coffea liberica* [coffee], Scrophulariaceae such as the genus *Verbascum*, for example the genera and species *Verbascum blattaria*, *Verbascum chaixii*, *Verbascum densiflorum*, *Verbascum lagurus*, *Verbascum longifolium*, *Verbascum lychnitis*, *Verbascum nigrum*, *Verbascum olympicum*, *Verbascum phlomoides*, *Verbascum phoenicum*,

25 *Verbascum pulverulentum* or *Verbascum thapsus* [mullein], Solanaceae such as the genera *Capsicum*, *Nicotiana*, *Solanum*, *Lycopersicon*, for example the genera and species *Capsicum annuum*, *Capsicum annuum* var. *glabriusculum*, *Capsicum frutescens* [pepper], *Capsicum annuum* [paprika], *Nicotiana tabacum*, *Nicotiana alata*, *Nicotiana attenuata*, *Nicotiana glauca*, *Nicotiana langsdorffii*, *Nicotiana obtusifolia*, *Nicotiana quadrivalvis*,

30 *Nicotiana repanda*, *Nicotiana rustica*, *Nicotiana sylvestris* [tobacco], Solanum tuberosum [potato], *Solanum melongena* [eggplant], *Lycopersicon esculentum*, *Lycopersicon lycopersicum*, *Lycopersicon pyriforme*, *Solanum integrifolium* or *Solanum lycopersicum* [tomato], Sterculiaceae, such as the genus *Theobroma*, for example the genus and species *Theobroma cacao* [cacao] or Theaceae, such as the genus *Camellia*, for example the genus and species *Camellia sinensis* [tea]. In particular preferred plants to be used as transgenic plants in accordance with the present invention are oil fruit crops which comprise large amounts of lipid compounds, such as peanut, oilseed rape, canola, sunflower,

safflower, poppy, mustard, hemp, castor-oil plant, olive, sesame, Calendula, Punica, evening primrose, mullein, thistle, wild roses, hazelnut, almond, macadamia, avocado, bay, pumpkin/squash, linseed, soybean, pistachios, borage, trees (oil palm, coconut, walnut) or crops such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper,

- 5 Tagetes, Solanaceae plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa or bushy plants (coffee, cacao, tea), SaNx species, and perennial grasses and fodder crops. Preferred plants according to the invention are oil crop plants such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, Calendula, Punica, evening primrose, pumpkin/squash, linseed, soybean, borage, trees (oil 10 palm, coconut). Especially preferred are sunflower, safflower, tobacco, mullein, sesame, cotton, pumpkin/squash, poppy, evening primrose, walnut, linseed, hemp, thistle or safflower. Very especially preferred plants are plants such as safflower, sunflower, poppy, evening primrose, walnut, linseed, or hemp.

- 15 Preferred mosses are Physcoimitrella or Ceratodon. Preferred algae are Isochrysis, Mantoniella, Ostreococcus or Cryptecodinium, and algae/diatoms such as Phaeodactylum or Thraustochytrium. More preferably, said algae or mosses are selected from the group consisting of: Emiliania, Shewanella, Physcomitrella, Thraustochytrium, Fusarium, Phytophthora, Ceratodon, Isochrysis, Aleurita, Muscaroides, Mortierella, Phaeodactylum, 20 Cryptecodinium, specifically from the genera and species Thallasiosira pseudonona, Euglena gracilis, Physcomitrella patens, Phytophthora infestans, Fusarium graminearum, Cryptecodinium cohnii, Ceratodon purpureus, Isochrysis galbana, Aleurita farinosa, Thraustochytrium sp., Muscaroides vialii, Mortierella alpina, Phaeodactylum tricornutum or Caenorhabditis elegans or especially advantageously Phytophthora infestans, Thallasiosira 25 pseudonona and Cryptecodinium cohnii.

Transgenic plants may be obtained by transformation techniques as elsewhere in this specification. Preferably, transgenic plants can be obtained by T-DNA-mediated transformation. Such vector systems are, as a rule, characterized in that they contain at 30 least the vir genes, which are required for the Agrobacterium-mediated transformation, and the sequences which delimit the T-DNA (T-DNA border). Suitable vectors are described elsewhere in the specification in detail.

Also encompassed are transgenic non-human animals comprising the vector or 35 polynucleotide of the present invention. Preferred non-human transgenic animals envisaged by the present invention are fish, such as herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna.

However, it will be understood that dependent on the non-human transgenic organism 40 specified above, further, enzymatic activities may be conferred to the said organism, e.g., by recombinant technologies. Accordingly, the present invention, preferably, envisages a non-human transgenic organism specified above which in addition to the polynucleotide of

the present invention comprises polynucleotides encoding such desaturases and/or elongases as required depending on the selected host cell. Preferred desaturases and/or elongases which shall be present in the organism are at least one enzyme selected from the group of desaturases and/or elongases or the combinations specifically recited
5 elsewhere in this specification (see above and Tables 3, 4 and 5).

Furthermore, the present invention encompasses a method for the manufacture of polyunsaturated fatty acids comprising:

- a) cultivating the host cell of the invention under conditions which allow for the production
10 of polyunsaturated fatty acids in said host cell;
- b) obtaining said polyunsaturated fatty acids from the said host cell.

The term " polyunsaturated fatty acids (PUFA)" as used herein refers to fatty acids comprising at least two, preferably, three, four, five or six, double bonds. Moreover, it is to
15 be understood that such fatty acids comprise, preferably from 18 to 24 carbon atoms in the fatty acid chain. More preferably, the term relates to long chain PUFA (LCPUFA) having from 20 to 24 carbon atoms in the fatty acid chain. Preferred unsaturated fatty acids in the sense of the present invention are selected from the group consisting of DGLA 20:3 (8,1 1,14), ARA 20:4 (5,8,1 1,14), iARA 20:4(8,1 1,14,17), EPA 20:5 (5,8,1 1,14,17), DPA 22:5
20 (4,7,10,13,16), DHA 22:6 (4,7,10,13,16,19), 20:4 (8,1 1,14,17), more preferably, arachidonic acid (ARA) 20:4 (5,8,1 1,14), eicosapentaenoic acid (EPA) 20:5 (5,8,1 1,14,17), and docosahexaenoic acid (DHA) 22:6 (4,7,10,13,16,19). Thus, it will be understood that most
25 preferably, the methods provided by the present invention pertaining to the manufacture of ARA, EPA or DHA. Moreover, also encompassed are the intermediates of LCPUFA which occur during synthesis. Such intermediates are, preferably, formed from substrates by the desaturase or elongase activity of the polypeptides of the present invention. Preferably, substrates encompass LA 18:2 (9,12), ALA 18:3(9,12,15), Eicosadienoic acid 20:2 (11,14),
Eicosatrienoic acid 20:3 (11,14,17)), DGLA 20:3 (8,1 1,14), ARA 20:4 (5,8,1 1,14), eicosatetraenoic acid 20:4 (8,1 1,14,17), Eicosapentaenoic acid 20:5 (5,8,1 1,14,17),
30 Docosahexapentanoic acid 22:5 (7,10,13,16,19).

The term " cultivating" as used herein refers maintaining and growing the host cells under culture conditions which allow the cells to produce the said polyunsaturated fatty acid, i.e. the PUFA and/or LCPUFA referred to above. This implies that the polynucleotide of the
35 present invention is expressed in the host cell so that the desaturase and/or elongase activity is present. Suitable culture conditions for cultivating the host cell are described in more detail below.

The term " obtaining" as used herein encompasses the provision of the cell culture including the host cells and the culture medium as well as the provision of purified or partially purified preparations thereof comprising the polyunsaturated fatty acids, preferably, ARA, EPA, DHA, in free or in -CoA bound form, as membrane phospholipids or as

triacylglyceride esters. More preferably, the PUFA and LCPUFA are to be obtained as triglyceride esters, e.g., in form of an oil. More details on purification techniques can be found elsewhere herein below.

- 5 The host cells to be used in the method of the invention are grown or cultured in the manner with which the skilled worker is familiar, depending on the host organism. Usually, host cells are grown in a liquid medium comprising a carbon source, usually in the form of sugars, a nitrogen source, usually in the form of organic nitrogen sources such as yeast extract or salts such as ammonium sulfate, trace elements such as salts of iron, manganese and magnesium and, if appropriate, vitamins, at temperatures of between 0°C and 100°C, preferably between 10°C and 60°C under oxygen or anaerobic atmosphere dependent on the type of organism. The pH of the liquid medium can either be kept constant, that is to say regulated during the culturing period, or not. The cultures can be grown batchwise, semibatchwise or continuously. Nutrients can be provided at the beginning of the
- 10 fermentation or administered semicontinuously or continuously: The produced PUFA or LCPUFA can be isolated from the host cells as described above by processes known to the skilled worker, e.g., by extraction, distillation, crystallization, if appropriate precipitation with salt, and/or chromatography. It might be required to disrupt the host cells prior to purification. To this end, the host cells can be disrupted beforehand. The culture medium to
- 15 be used must suitably meet the requirements of the host cells in question. Descriptions of culture media for various microorganisms which can be used as host cells according to the present invention can be found in the textbook "Manual of Methods for General Bacteriology" of the American Society for Bacteriology (Washington D.C , USA, 1981). Culture media can also be obtained from various commercial suppliers. All media
- 20 components are sterilized, either by heat or by filter sterilization. All media components may be present at the start of the cultivation or added continuously or batchwise, as desired. If the polynucleotide or vector of the invention which has been introduced in the host cell further comprises an expressible selection marker, such as an antibiotic resistance gene, it might be necessary to add a selection agent to the culture, such as a antibiotic in order to
- 25 maintain the stability of the introduced polynucleotide. The culture is continued until formation of the desired product is at a maximum. This is normally achieved within 10 to 160 hours. The fermentation broths can be used directly or can be processed further. The biomass may, according to requirement, be removed completely or partially from the fermentation broth by separation methods such as, for example, centrifugation, filtration,
- 30 decanting or a combination of these methods or be left completely in said broth. The fatty acid preparations obtained by the method of the invention, e.g., oils, comprising the desired PUFA or LCPUFA as triglyceride esters are also suitable as starting material for the chemical synthesis of further products of interest. For example, they can be used in combination with one another or alone for the preparation of pharmaceutical or cosmetic
- 35 compositions, foodstuffs, or animal feeds. Chemically pure triglycerides comprising the desired PUFA or LCPUFA can also be manufactured by the methods described above. To this end, the fatty acid preparations are further purified by extraction, distillation,
- 40

crystallization, chromatography or combinations of these methods. In order to release the fatty acid moieties from the triglycerides, hydrolysis may be also required. The said chemically pure triglycerides or free fatty acids are, in particular, suitable for applications in the food industry or for cosmetic and pharmacological compositions.

5

Moreover, the present invention relates to a method for the manufacture of poly-unsaturated fatty acids comprising:

- a) cultivating the non-human transgenic organism of the invention under conditions which allow for the production of poly-unsaturated fatty acids in said non-human transgenic organism; and
- b) obtaining said poly-unsaturated fatty acids from the said non-human transgenic organism.

Further, it follows from the above that a method for the manufacture of an oil, lipid or fatty acid composition is also envisaged by the present invention comprising the steps of any one of the aforementioned methods and the further step of formulating PUFA or LCPUFA as oil, lipid or fatty acid composition. Preferably, said oil, lipid or fatty acid composition is to be used for feed, foodstuffs, cosmetics or medicaments. Accordingly, the formulation of the PUFA or LCPUFA shall be carried out according to the GMP standards for the individual envisaged products. For example, an oil may be obtained from plant seeds by an oil mill. However, for product safety reasons, sterilization may be required under the applicable GMP standard. Similar standards will apply for lipid or fatty acid compositions to be applied in cosmetic or pharmaceutical compositions. All these measures for formulating oil, lipid or fatty acid compositions as products are comprised by the aforementioned manufacture.

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The term "oil" refers to a fatty acid mixture comprising unsaturated and/or saturated fatty acids which are esterified to triglycerides. Preferably, the triglycerides in the oil of the invention comprise PUFA or LCPUFA as referred to above. The amount of esterified PUFA and/or LCPUFA is, preferably, approximately 30%, a content of 50% is more preferred, a content of 60%, 70%, 80% or more is even more preferred. The oil may further comprise free fatty acids, preferably, the PUFA and LCPUFA referred to above. For the analysis, the fatty acid content can be, e.g., determined by GC analysis after converting the fatty acids into the methyl esters by transesterification. The content of the various fatty acids in the oil or fat can vary, in particular depending on the source. The oil, however, shall have a non-naturally occurring composition with respect to the PUFA and/or LCPUFA composition and content. It will be understood that such a unique oil composition and the unique esterification pattern of PUFA and LCPUFA in the triglycerides of the oil shall only be obtainable by applying the methods of the present invention specified above. Moreover, the oil of the invention may comprise other molecular species as well. Specifically, it may comprise minor impurities of the polynucleotide or vector of the invention. Such impurities, however, can be detected only by highly sensitive techniques such as PCR.

The contents of all references cited throughout this application are herewith incorporated by reference in general and with respect to their specific disclosure content referred to above.

FIGURES

5

Figure 1 shows a schematical figure of the different enzymatic activities leading to the production of ARA, EPA and DHA.

10 Figure 2 shows a yeast expression experiment with feeding of 22:5n-3 in the presence (A) and absence (B) of d4Des(Eh)

Figure 3 shows a yeast expression experiment with feeding of 20:3n-3 in the presence (A) and absence (B) of d8Des(Eh)

15 Figure 4 shows a yeast expression experiment with feeding of 18:3n-3 in the presence (A) and absence (B) of d9Elo(Eh)

Figure 5 shows a yeast expression experiment with feeding of 18:3n-6 (GLA) and 18:4n-3 (SDA) in the presence (A) and absence (B) of d5Elo(Eh)

20

Figure 6 shows a yeast expression experiment with feeding of 20:4n-6 (ARA) and 20:5n-3 (EPA) in the presence (A) and absence (B) of d5Elo(Eh)

25

Figure 7 shows the expression of d9Elo(Eh) in seeds of two Arabidopsis events. As control seeds not expressing d9Elo(Eh) are shown (WT).

Figure 8 shows the Acyl-CoA analysis of mature Arabidopsis seeds from both events expressing the d9Elo(Eh) in comparison to seeds not expressing d9Elo(Eh) (CoLO)).

30

Figure 9 shows the expression of d9Elo(Eh), d8Des(Eh) and d5Des(Eh) in seeds of various Arabidopsis events.

35

Figure 10 shows gas chromatographic analysis of mature Arabidopsis seeds transformed with the construct OstELO5EmD4. Peaks were quantified and listed in the table below. The products of d5Elo(Ot) and d4Des(Eh) activity are 22:6n-3 (DHA).

Figure 11 is a comparison between two d4-desaturases (Tc and Eh) showing that d4Des(Eh) is different from known d4-desaturases in producing a high ratio of DHA:DPA.

40

Figure 12 shows the expression of d5Elo(Eh) in seeds of various Arabidopsis events.

Figure 13 is a comparison between three different d6-desaturases and the substrate specificity

ofd5Des(Eh).

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited

5 throughout this application, as well as the figures, are incorporated herein by reference.

EXAMPLES

Example 1: Organism and culture conditions

10 *Emiliana huxleyi* was grown as described in Sciandra et al. (2003) Marine Ecology Progress Series 261 :111-122 with following conditions:

Growth in 50 ml inconical flasks using K/2 medium (Keller et al. (1987) Journal of Phycology 23:633-638). The flasks were placed in a growth chamber at a temperature of $17 \pm 0.1^{\circ}\text{C}$ under 14L10D irradiance. Light was provided by fluorescent lamps giving a photon

15 fluxdensity (400 to 700 nm) of 170 $\mu\text{ mol photon m}^{-2}\text{ s}^{-1}$.

Example 2: Cloning of novel desaturase and elongase sequences

RNA from cells grown as described under Example 1 was extracted using the RNA-extraction Kit from Qiagen, a RACE-library was generated using the RACE-Kit from

20 Clontech. From the RACE-library sequences for desaturase and elongases were amplified with PCR using following primer pairs and PCR conditions.

PCR reaction (50 $\mu\text{ L}$):

5,00 $\mu\text{ L}$ Template cDNA

25 5,00 $\mu\text{ L}$ 10x Puffer (Advantage-Polymerase)+ 25mM MgCl₂

5,00 $\mu\text{ L}$ 2mM dNTP

1,25 $\mu\text{ L}$ je Primer (10 pmol/ $\mu\text{ L}$)

0,50 $\mu\text{ L}$ Advantage-Polymerase

The Advantage polymerase mix from Clontech was used.

30 Reaction conditions of the PCR:

Annealing: 1 min 55 $^{\circ}\text{C}$

Denaturation: 1 min 94 $^{\circ}\text{C}$

Elongation: 2 min 72 $^{\circ}\text{C}$

Cycles: 35

35

Primer pairs used in PCR:

Name	Primer pair (5' orientation)	SEQ ID NO.
Eh4ff	CCATGGGAGGCGCCGGCGAG	11
Eh4rv	CTAGTCCGCCTTGAGGTTCTC	12
Eh5ff	ACCATGTGCAAGGCGAGCGGCCT	13
Eh5rv	TCACCAATCATGAGGAAGGT	14
Eh8ff	CCATGGGCAAGGGCGGCAACGC	15

Eh8rv	GGGCAGAGATGCCGCACTAG	16
Eh9ff	ACCATGCTCGATCGCGCCTCGTC	17
Eh9rv	TCACAGCGCCTTGCGGGTAGC	18

The PCR reactions resulted in following polynucleotide sequences:

Gene	Activity	Length in bp	SEQ ID NO.
D4Des(Eh)	D4-desaturase	1280	5
D8Des(Eh)	D8-desaturase	1256	1
D9Elo(Eh)	D9-elongase	804	3
D5Elo(Eh)	Multi-elongase	921	7

A list of identified full-length coding sequences is shown in Table 1.

5

Table 1: List of full-length coding sequences and deduced amino acid sequences

SEQ ID NO:	Gene	Coding sequence in bp	Amino acid sequence
1	D8Des(Eh)	1254	417
3	D9Elo(Eh)	801	266
5	D4Des(Eh)	1278	425
7	D5Elo(Eh)	918	305

Open reading frames as shown in Table 1 were cloned into the pESC(Leu) vector from

10 Stratagene according to manufactures reaction conditions. Reactions were transformed into E. coli DH5 α and plasmid DNA was isolated. The plasmids pESC-d4Des(Eh), pESC-d8Des(Eh), pESC-d9Elo(Eh), pESC-d δ Elo(Eh) were then used for yeast transformation.

Example 3: Yeast Transformation and Growth Conditions

15 S. cerevisiae strain INVSC from Invitrogen was transformed with the constructs pESC-d4Des(Eh), pESC-d δ Des(Eh), pESC-d9Elo(Eh), pESC-d5Elo(Eh) and pESC using the S. C. EasyComp Transformation Kit (Invitrogen, Carlsbad, California) with selection on leucine-deficient medium.

Yeast were grown after transformation in complete medium containing all amino acids and
20 nucleotides. Then yeast were plated on different medium containing either the complete medium (SD) or the complete medium lacking leucine (SD-Leu). Only yeast containing pESC-d4Des(Eh), pESC-d δ Des(Eh), pESC-d9Elo(Eh), pESC-d5Elo(Eh) or pESC vector can grow on this medium.

25 Example 4: Functional expression of desaturases and elongases in yeast and gas chromatographic analysis.

Yeast cells containing the respective pESC plasmids as prepared above were incubated 12

h in liquid DOB-U medium at 28 °C, 200 rpm inkubiert and than additional 12 h in induction medium (DOB-U+2% (w/v) galactose + 2% (w/v) raffinose). To the induction medium 250 µ M of the respective fatty acids were added to check for enzyme activity and specificity.

5 Yeast cells were analyzed as following:

Yeast cells from induction medium were harvested by centrifugation (100 x g, 5 min, 20°C) and washed with 100 mM NaHCCb, pH 8,0, to remove residual fatty acids. From the yeast pellet a total extract of fatty acid methylesters (FAME) was generated by adding 2 ml 1 N methanolic sulfuric acid and 2% (v/v) Dimethoxypropan for 1 h at 80°C. FAME were
10 extracted two times with Petrolether (PE). Not derivased fatty acids were removed by washing with 2 ml 100 mM NaHCO₃, pH 8,0 and 2 ml Aqua dest. The PE-phases were dried with Na₂SO₄ and eluted in 100 µl PE. The samples were then separated with a DB-23-column (30 m, 0,25 mm, 0,25 µm, Agilent) in a Hewlett-Packard 6850-machine with FID
15 using following conditions: oven temperature 50°C to 250°C with a rate of 5°C/min and finally 10 min at 250°C.

The identification of the fatty acids was done using the retention times of known fatty acid standards (Sigma). The method is described e.g. in Napier and Michaelson, 2001 ,Lipids. 36(8):761-766; Sayanova et al., 2001 , Journal of Experimental Botany. 52(360):1 581 -1585,
20 Sperling et al., 2001 , Arch. Biochem. Biophys. 388(2):293-298 und Michaelson et al., 1998, FEBS Letters. 439(3):215-218.

Example 5: Functional characterization of d4Des(Eh).

As described above d4Des(Eh) was functionally characterized in yeast. The result of the
25 analysis is shown in Figure 2. Yeast transformed with pESC-d4Des(Eh) was compared to yeast transformed with pESC (control) while feeding the yeast cells with the fatty acid DPA 22:5n-3. Based on this comparison pESC-d4Des(Eh) exhibits d4-desaturase activity as in the control no 22:6 is observed. Therefore d4Des(Eh) is a functional d4-desaturase.

30 Example 6: Functional characterization of d8Des(Eh).

As described above d8Des(Eh) was functionally characterized in yeast. The result of the analysis is shown in Figure 3. Yeast transformed with pESC-d8Des(Eh) was compared to yeast transformed with pESC (control) while feeding the fatty acid 20:3n-3. Based on this comparison a new fatty acid was formed compared to the control, which is 20:4n-3. The
35 formation of this fatty acid proves that d8Des(Eh) was functionally expressed and has d8-desaturase activity. The conversion rate of 20:3n-3 to 20:4n-3 was 5%.

Example 7: Functional characterization of d9Elo(Eh).

As described above d9Elo(Eh) was functionally characterized in yeast. The result of the
40 analysis is shown in Figure 4. Yeast transformed with pESC-d9Elo(Eh) was compared to yeast transformed with pESC (control) while feeding the fatty acids 18:3n-3 (ALA) or 18:2 (LA). Based on this comparison a new fatty acid was formed compared to the control, which

is 20:3n-3 or 20:2n-6, respectively. The formation of these fatty acids proves that d9Elo(Eh) was functionally expressed and has d9-elongase activity. The conversion rate of 18:3n-3 to 20:3n-3 was 17%, the conversion rate of 18:2n-6 to 20:2n-6 was 49%.

5 Example 8: Functional characterization of d5Elo(Eh).

As described above d5Elo(Eh) was functionally characterized in yeast. The result of the analysis is shown in Figures 5 and 6. Yeast transformed with pESC-d5Elo(Eh) was compared to yeast transformed with pESC (control) while feeding the fatty acids 18:3n-6 (GLA), 18:4 (SDA) or 20:4n-6 (ARA), 20:5n-3 (EPA), respectively. Based on this

10 comparison new fatty acids formation was observed when compared to the control, which is 20:3n-6 or 20:4n-3 when fed GLA or SDA and 22:4n-6 or 22:5n-3 when fed ARA or EPA, respectively. The formation of these fatty acids proves that d5Elo(Eh) was functionally expressed and has d5-elongase activity. The conversion rate of GLA was 13%, the conversion rate of 18:4n-3 was 30%, the conversion rate of ARA was 38% and the 15 conversion rate of EPA was 30%. Surprisingly the elongase used a wide variety of substrates of elongation. The specification indicates a multifunctional elongase activity with higher specificities for omega3 fatty acids.

Example 9: Expression of novel elongases from *Emiliana huxleyi* in plants.

20

The novel desaturases and elongases were cloned into a plant transformation vector as described in WO2003/093482, WO2005/083093 or WO2007/093776. Exemplary suitable combinations of genes are described in Table 2, 3 and 4.

25 Table 2: Gene combinations for the production of ARA.

Gene	Aktivität	SEQ ID NO:
D6Des(Ot)	Δ6-Desaturase	19
D6Elo(Pp)	Δ6-Elongase	21
D5Des(Eh)	Δ5-Desaturase	9
D12Des(Ps)	Δ12-Desaturase	23
D6Elo(Tp)	Δ6-Elongase	25
D8Des(Eh)	Δ8-Desaturase	1
D9Elo(Eh)	Δ9-Elongase	3

Table 3: Gene combinations for the production of EPA.

Gene	Aktivität	SEQ ID NO:
D6Des(Ot)	Δ6-Desaturase	19
D5Elo(Eh)	Δ5-Elongase	7
D5Des(Eh)	Δ5-Desaturase	9
D12Des(Ps)	Δ12-Desaturase	23

D6Elo(Tp)	$\Delta 6$ -Elongase	25
$\omega 3$ -Des(Pi)	Omega 3-Desaturase	27
D15Des(Cp)	$\Delta 15$ -Desaturase	29
D8Des(Eh)	$\Delta 8$ -Desaturase	1
D9Elo(Eh)	$\Delta 9$ -Elongase	3

Table 4: Gene combinations for the production of DHA.

Gene	Aktivität	SEQ ID NO:
D6Des(Ot)	$\Delta 6$ -Desaturase	19
D5Elo(Eh)	$\Delta 5$ -Elongase	7
D5Des(Eh)	$\Delta 5$ -Desaturase	9
D12Des(Ps)	$\Delta 12$ -Desaturase	23
D6Elo(Tp)	$\Delta 6$ -Elongase	25
$\omega 3$ -Des(Pi)	Omega 3-Desaturase	27
D15Des(Cp)	$\Delta 15$ -Desaturase	29
D5Elo(Ot)	$\Delta 5$ -elongase	31
D4Des(Eh)	$\Delta 4$ -desaturase	5
D8Des(Eh)	$\Delta 8$ -Desaturase	1
D9Elo(Eh)	$\Delta 9$ -Elongase	3

Based on the gene combinations as described in Table 2, Table 3 or Table 4 following combinations were designed

- 5 • AP2: LuCnl-d5Des(Eh)_LuCnl-d8Des8Eh)_Napin- $\omega 3$ Des(Pi)_Napin-d12Des(Ps)_LuCnl-d9Elo(Eh)
- OstELO5EmD4: VfUSP-d6Elo(Pp)_LuCnl-d5Des8Tc)_VfSBP-d6Des(Ot)_Napin- $\omega 3$ Des(Pi)_Napin-d12Des(Ps)_LuCnl-d5Elo(Ot)_LuCnl-d4Des(Eh)
- OstELO5TcD4: VfUSP-d6Elo(Pp)_LuCnl-d5Des8Tc)_VfSBP-d6Des(Ot)_Napin- $\omega 3$ Des(Pi)_Napin-d12Des(Ps)_LuCnl-d5Elo(Ot)_LuCnl-d4Des(Tc)

Transgenic rapeseed lines were generated as described in Deblaere et al, 1984, Nucl. Acids. Res. 13, 4777-4788 and seeds of transgenic rapeseed plants are analyzed as described in Qiu et al. 2001, J. Biol. Chem. 276, 31561-31566.

- 15 Transgenic Arabidopsis plants were generated as described in Bechtholdt et al. 1993 CR. Acad. Sci. Ser. III ScL Vie., 316, 1194-1 199. Seeds of transgenic Arabidopsis plants expressing d9Elo(Eh) by using the seed-specific promoter Glycinin from soybean (Lelievre et al. (1992) Plant Physiol 98:387-391) were analyzed by gas chromatography (Figure 7).
- 20 Compared to non-transgenic control plants (WT) there are changes in the fatty acid profile, proving that d9Elo(Eh) was functionally expression in seeds. The major shifts in the fatty acid profile is directed to a 10fold increase in the fatty acid 20:2n-6 and 20:3n-3 (Figure 7). Therefore d9Elo(Eh) exhibits a $\Delta 9$ -elongase activity, which is consistent with the yeast characterization. Further, the levels of 18:2 and ALA in the transgenic events expressing

d9Elo(Eh) are lowered compared to WT, as these fatty acids are direct substrates for the d9Elo(Eh). Further, the endogenous elongation system in the plant is unchanged as levels of 20:1 and 22:1 are similar between transgenic plants expression d9Elo(Eh) and WT control. This indicates that the expression of d9Elo(Eh) does not disturb endogenous

5 elongation process, but delivers additional activity.

To further prove the activity of d9Elo(Eh) expressed in seeds of *Arabidopsis thaliana* AcylCoA-measurements were done. Substrates and products of the d9Elo(Eh) elongation reaction are AcylCoA-esters, which are then further incorporated into triacylglycerides (oil).

10 The analysis of the acylCoA-pool reveals the formation and flux of the elongation reaction. Figure 8 summarizes the AcylCoA measurements for *Arabidopsis* event expressing d9Elo(Eh) in comparison to controls not expressing d9Elo(Eh) (ColO). The change in the chromatogram is indicated by a star. At this position a massive amount of 20:2n-6 is detected, which is much lower in the control. The conditions for separation of the fatty acid

15 CoA-esters does not allow the detection of 20:3n-3 as this CoA ester is not separated from 18:3CoA.

The massive occurrence of 20:2n-6-CoA proves the expression of d9Elo(Eh) as this is the direct product of its enzymatic activity.

20 Further, transgenic *Arabidopsis* lines have been generated to validate the activity of d8Des(Eh) and d5Des(Eh). Vector AP2 has been constructed according to standard molecular biology steps as described in WO2003/093482, WO2005/083093, WO2007/093776 or WO2009/016202 and transformed into *Arabidopsis thaliana* as described above. Analysis of transgenic seeds is shown in Fig. 9. The products of d9Elo(Eh) are 20:2 and 20:3n-3.

25 Further, transgenic *Arabidopsis* lines have been generated to validate the activity of d4Des(Eh). Construct OstELO5EmD4 was transformed into *Arabidopsis* as described above and seeds of a number of individual lines have been analyzed by gas chromatography (Fig. 10). The activity of d4Des(Eh) is demonstrated by the formation of DHA 22:6 (last column). All lines show the

30 production of DHA with levels of up to 4.7%. Of special interest is the ratio of DHA to DPA. Surprisingly the ratio of d4Des(Eh) is much higher than in d4-desaturases known in the art. A comparison against the d4-desaturase from *Thraustochytrium* ssp. of WO2002/026946 is shown in Fig. 11. The enzyme from *Thraustochytrium* ssp. showed so far highest levels of DHA (WO2005/083093), but with an unfavorable ratio of DPA to DHA. A high ratio of DHA:DPA is for

35 the commercial use of such oils of importance.

Further, transgenic *Arabidopsis* lines have been generated to validate the activity of d5Elo(Eh). Construct EmELO5TcD4 was transformed into *Arabidopsis* as described above and seeds of a number of individual lines have been analyzed by gas chromatography (Fig. 12). The activity of

40 d5Elo(Eh) is demonstrated by the formation of DPA 22:5 and DHA 22:6. Most lines show the production of these two fatty acids, proofing that d5Elo(Em) is functionally expressed in the seeds.

Further, transgenic *Arabidopsis* lines have been generated to validate the activity and substrate specificity of d5Des(Eh). For this purpose two $\Delta 6$ -desaturases were selected based on their different substrate specificity. The borage $\Delta\Theta$ is expected to use phosphatidylcholin-18:2 as substrate (WO96/21022), whereas the *Ostreococcus* $\Delta 6$ (Ostr $\Delta\Theta$) uses Acyl-CoA ester (WO2005/012316). In combination with the d6-elongase from *Physcomitrella patens* (WO2001/059128) both d6-desaturases produce DGLA or 20:4n-3, respectively. The ratio of ARA to EPA is for the borage $\Delta\Theta$ 2.9, for the Ostr $\Delta\Theta$ 2.3. It is noted that the use of Ostr $\Delta\Theta$ results in 3-4 times higher levels of products compared to the borage $\Delta\Theta$. The further combination of the d5Des(Eh) resulted in the production of ARA and EPA, demonstrating the functionality of the d5Des(Eh). The conversion of d5Des(Eh) of DGLA to ARA is 29% (borage $\Delta\Theta$) or 47% (Ostr $\Delta\Theta$). For 20:4n-3 to EPA it is 33% (borage $\Delta\Theta$) or 20% (Ostr $\Delta\Theta$).

Based on these results it is concluded that for Acyl-CoA substrates d5Des(Eh) is specific for the omega θ fatty acid DGLA. This is a novel substrate specificity not observed in the state of the art d5-desaturases.

Reference List

- Arondel,V., Lemieux,B., Hwang,I., Gibson,S., Goodman,H.M., and Somerville,C.R. (1992). Map-based cloning of a gene controlling omega-3 fatty acid desaturation in Arabidopsis. 5 Science 258, 1353-1355.
- Broadwater,J.A., Whittle,E., and Shanklin,J. (2002). Desaturation and hydroxylation. Residues 148 and 324 of Arabidopsis FAD2, in addition to substrate chain length, exert a major influence in partitioning of catalytic specificity. J. Biol. Chem. 277, 15613-15620.
- Broun,P., Shanklin,J., Whittle,E., and Somerville,C. (1998b). Catalytic plasticity of fatty acid 10 modification enzymes underlying chemical diversity of plant lipids. Science 282, 1315-1317.
- Calvo,A.M., Gardner,H.W., and Keller,N.P. (2001). Genetic connection between fatty acid metabolism and sporulation in *Aspergillus nidulans*. J. Biol. Chem. 276, 25766-25774.
- Knutzon,D.S., Thurmond,J.M., Huang,Y.S., Chaudhary,S., Bobik,E.G., Jr., Chan,G.M., Kirchner,S.J., and Mukerji,P. (1998). Identification of Delta5-dehydratase from *Mortierella 15 alpina* by heterologous expression in Bakers' yeast and canola. J. Biol. Chem. 273, 29360-29366.
- Mantle,P.G. and Nisbet,L.J. (1976). Differentiation of *Claviceps purpurea* in axenic culture. J. Gen. Microbiol. 93, 321-334.
- Mey,G., Oeser,B., Lebrun,M.H., and Tudzynski,P. (2002). The biotrophic, non- 20 appressorium-forming grass pathogen *Claviceps purpurea* needs a Fus3/Pmk1 homologous mitogen-activated protein kinase for colonization of rye ovarian tissue. Mol. Plant Microbe Interact. 15, 303-312.
- Okuley,J., Lightner,J., Feldmann,K., Yadav,N., Lark,E., and Browse,J. (1994). Arabidopsis FAD2 gene encodes the enzyme that is essential for polyunsaturated lipid synthesis. Plant 25 Cell 6, 147-158.
- Qi,B., Fraser,T., Mugford,S., Dobson,G., Sayanova,O., Butler,J., Napier,J.A., Stobart,A.K., and Lazarus,C.M. (2004). Production of very long chain polyunsaturated omega-3 and omega-6 fatty acids in plants. Nat. Biotechnol. 22, 739-745.
- Qiu,X., Hong,H., and McKenzie,SL. (2001) Identification of a Delta 4 fatty acid desaturase 30 from *Thraustochytrium* sp. involved in the biosynthesis of docosahexanoic acid by heterologous expression in *Saccharomyces cerevisiae* and *Brassica juncea*. J Biol Chem 276, 31561-6.
- Shanklin,J. and Cahoon,E.B. (1998). DESATURATION AND RELATED MODIFICATIONS OF FATTY ACIDS1 . Annu. Rev. Plant Physiol Plant Mol. Biol. 49, 611-641 .
- Tudzynski,P., Correia,T., and Keller,U. (2001). Biotechnology and genetics of ergot 35 alkaloids. Appl. Microbiol. Biotechnol. 57, 593-605.

All references cited in this specification are herewith incorporated by reference with respect to their entire disclosure content and the disclosure content specifically mentioned in this 40 specification.

CLAIMS

1. A polynucleotide comprising a nucleic acid sequence elected from the group consisting of:
 - 5 a) a nucleic acid sequence having a nucleotide sequence as shown in SEQ ID NOs: 1, 3, 5, 7 or 9;
 - b) a nucleic acid sequence encoding a polypeptide having an amino acid sequence as shown in SEQ ID NOs: 2, 4, 6, 8 or 10;
 - c) a nucleic acid sequence being at least 70% identical to the nucleic acid sequence of a) or b), wherein said nucleic acid sequence encodes a polypeptide having desaturase or elongase activity;
 - 10 d) a nucleic acid sequence encoding a polypeptide having desaturase or elongase activity and having an amino acid sequence which is at least 82% identical to the amino acid sequence of any one of a) to c); and
 - e) a nucleic acid sequence which is capable of hybridizing under stringent conditions to any one of a) to d), wherein said nucleic acid sequence encodes a polypeptide having desaturase or elongase activity.
2. The polynucleotide of claim 1, wherein said polynucleotide further comprises an expression control sequence operatively linked to the said nucleic acid sequence.
- 20 3. The polynucleotide of claim 1 or 2, wherein said polynucleotide further comprises a terminator sequence operatively linked to the nucleic acid sequence.
- 25 4. A vector comprising the polynucleotide of any one of claims 1 to 3.
5. A host cell comprising the polynucleotide of any one of claims 1 to 3 or the vector of claim 4.
- 30 6. A method for the manufacture of a polypeptide encoded by a polynucleotide of any one of claims 1 to 3 comprising
 - a) cultivating the host cell of claim 5 under conditions which allow for the production of the said polypeptide; and
 - b) obtaining the polypeptide from the host cell of step a).
- 35 7. A polypeptide encoded by the polynucleotide of any one of claims 1 to 3 or which is obtainable by the method of claim 6.
8. A non-human transgenic organism comprising the polynucleotide of any one of claims 1 to 3 or the vector of claim 4
- 40 9. The non-human transgenic organism of claim 8, which is a plant, plant part, or plant

seed.

10. A method for the manufacture of polyunsaturated fatty acids comprising:
- cultivating the host cell of claim 5 under conditions which allow for the production
5 of polyunsaturated fatty acids in said host cell; and
 - obtaining said polyunsaturated fatty acids from the said host cell.

11. A method for the manufacture of polyunsaturated fatty acids comprising:
- cultivating the non-human transgenic organism of claim 8 or 9 under conditions
10 which allow for the production of polyunsaturated fatty acids in said non-human transgenic organism; and
 - obtaining said polyunsaturated fatty acids from the said non-human transgenic organism.

15 12. The method of claim 10 or 11, wherein said poly-unsaturated fatty acid is arachidonic acid (ARA), eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA).

13. A method for the manufacture of an oil, lipid or fatty acid composition comprising the steps of the method of any one of claims 10 to 12 and the further step of formulating
20 the polyunsaturated fatty acid as oil, lipid or fatty acid composition.

14. The method of claim 13, wherein said oil, lipid or fatty acid composition is to be used for feed, foodstuffs, cosmetics or medicaments.

25 15. An Antibody or a fragment thereof which specifically recognizes the polypeptide of claims 1 to 3.

Figure 1 of 13: Schematic figure of the different enzymatic activities leading to the production of ARA, EPA and DHA.

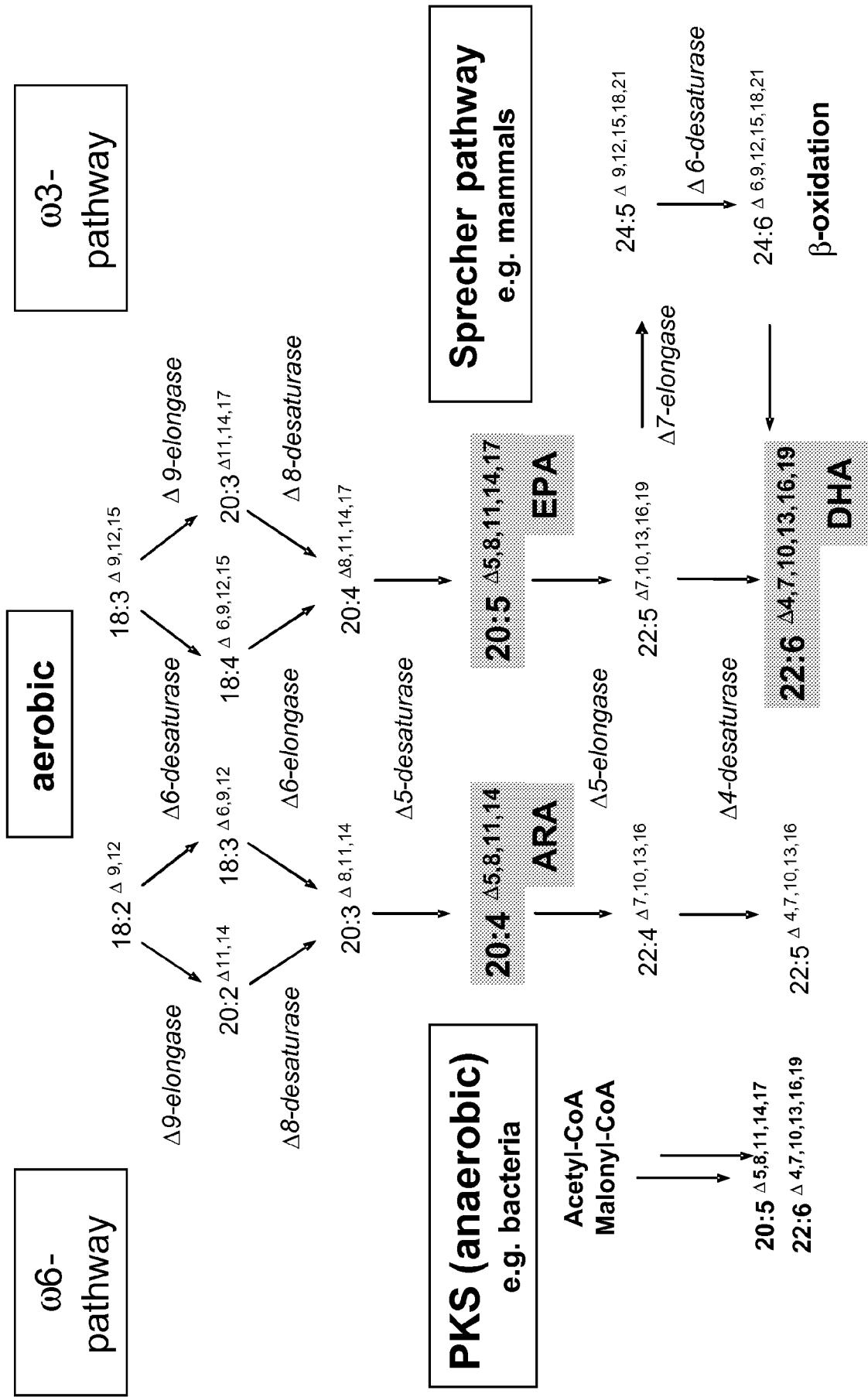


Figure 2 of 13: Gas chromatograph of yeast expression experiment with feeding of 22:5n-3 in the presence (A) and absence (B) of d4Des(Eh). The arrow indicates the formation of 22:6, the product of d4Des(Eh) activity.

Expression in yeast

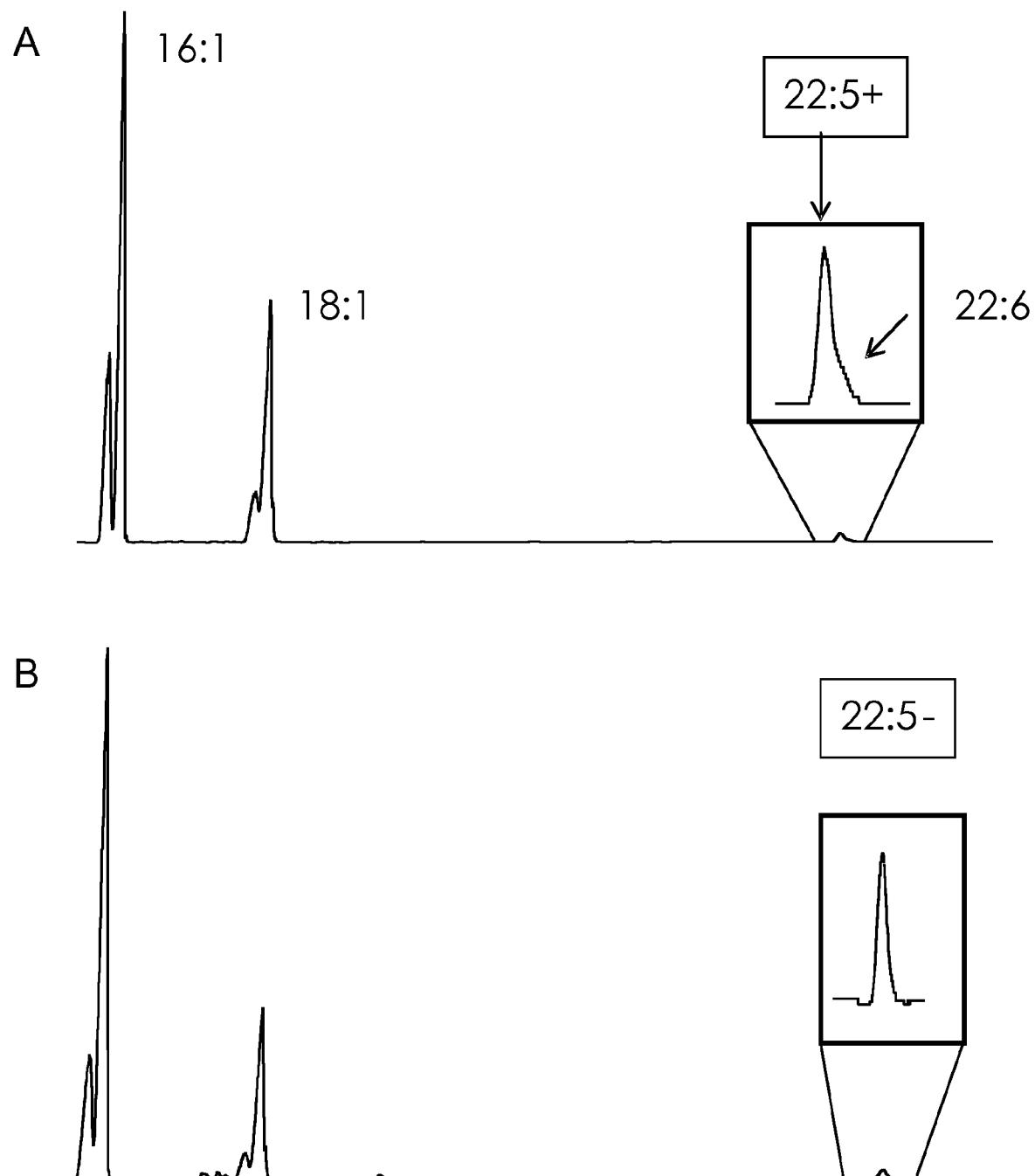


Figure 3 of 13: Gas chromatograph of yeast expression experiment with feeding of 20:3n-3 in the presence (A) and absence (B) of d8Des(Eh). The arrow indicates the formation of 20:4n-3, the product of d8Des(Eh) activity.

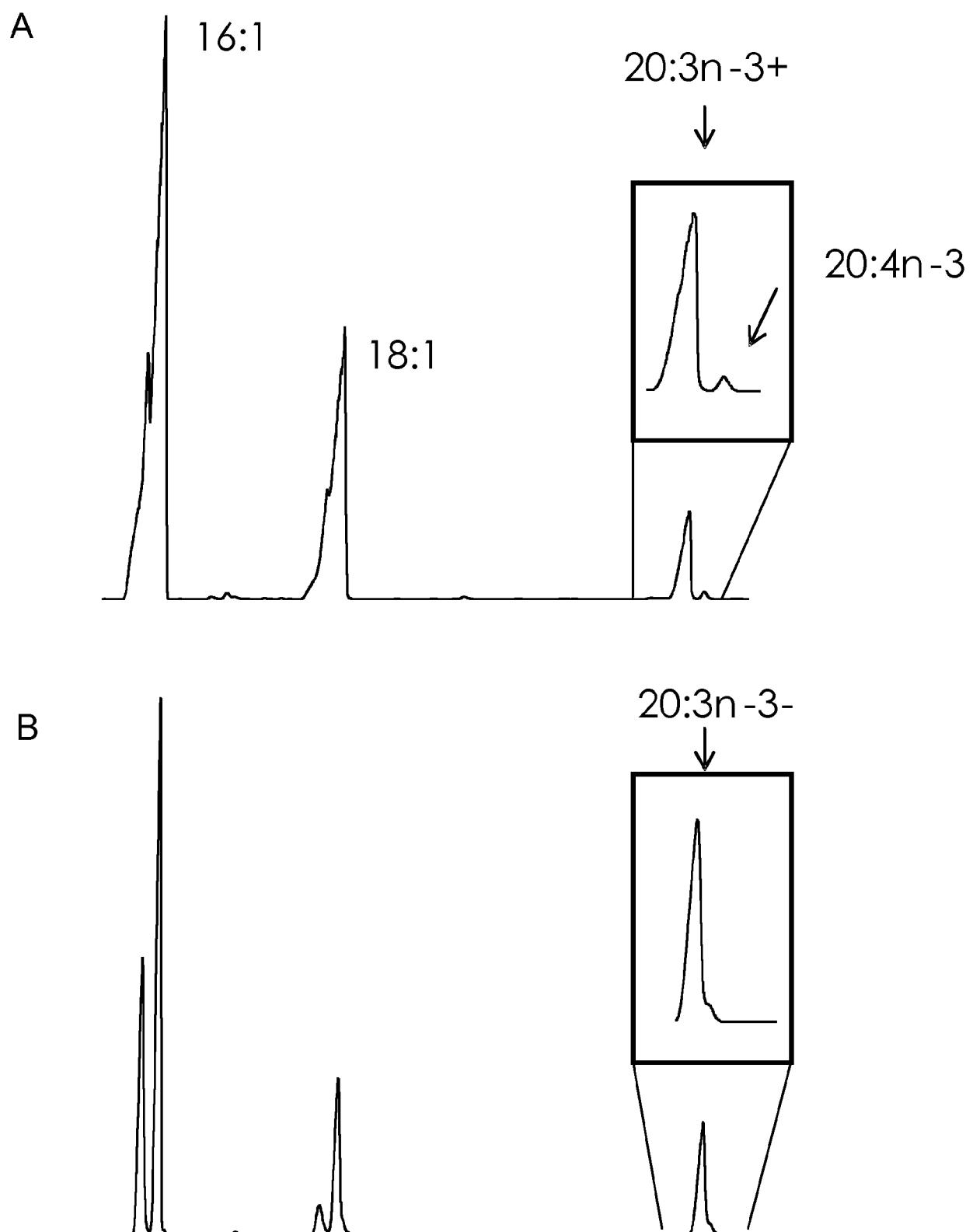


Figure 4 of 13: Gas chromatograph of yeast expression experiment with feeding of 18:3n-3 (ALA) in the presence (A) and absence (B) of d9Elo(Eh). The arrow indicates the formation of 20:3n-3, the product of d9Elo(Eh) activity.

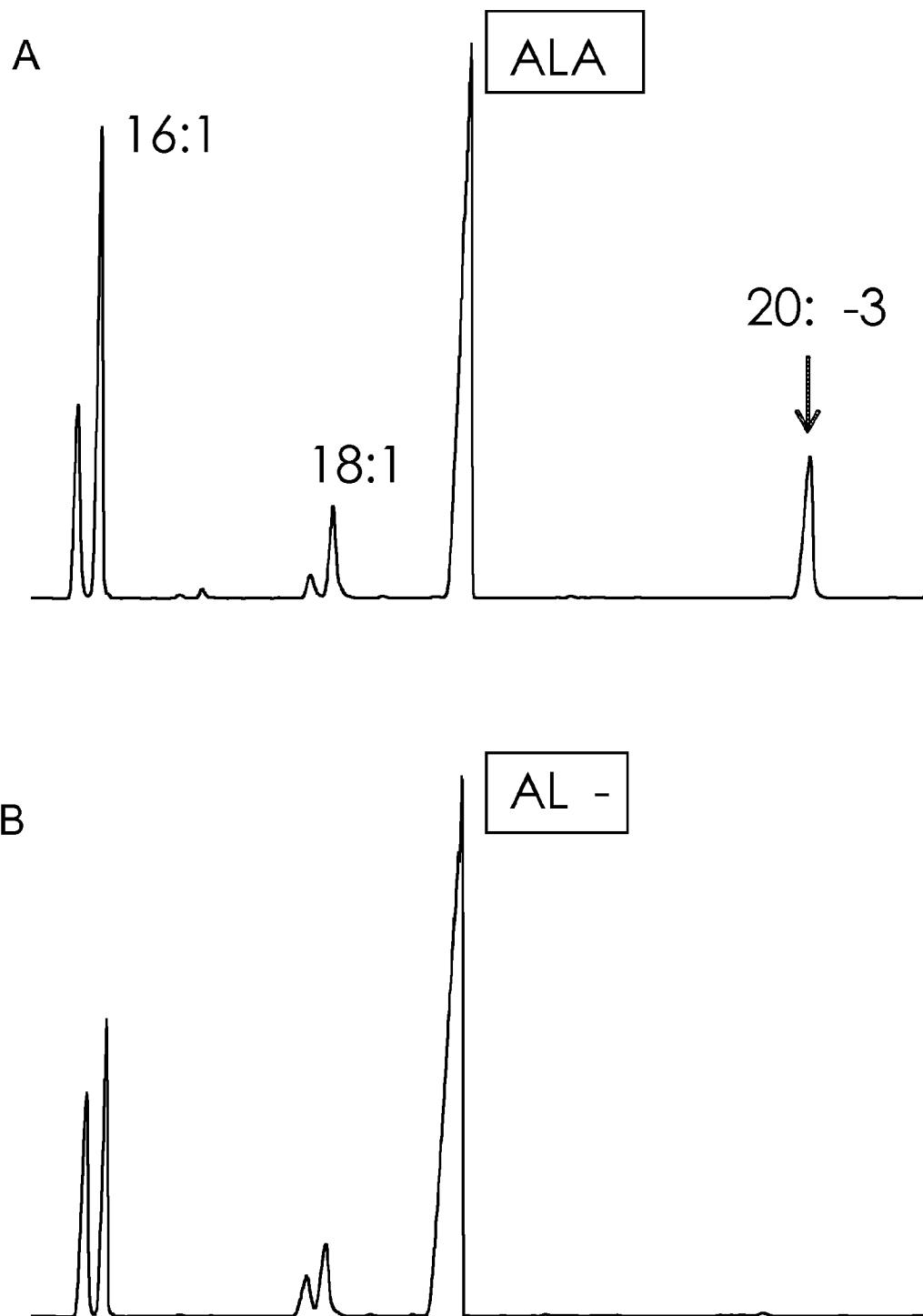


Figure 5 of 13: Gas chromatograph of yeast expression experiment with feeding of 18:3n-6 (GLA) and 18:4n-3 (SDA) in the presence (A) and absence (B) of d5Elo(Eh). The arrow indicates the formation of 20:3n-6 or 20:4n-3, respectively, the products of d5Elo(Eh) activity

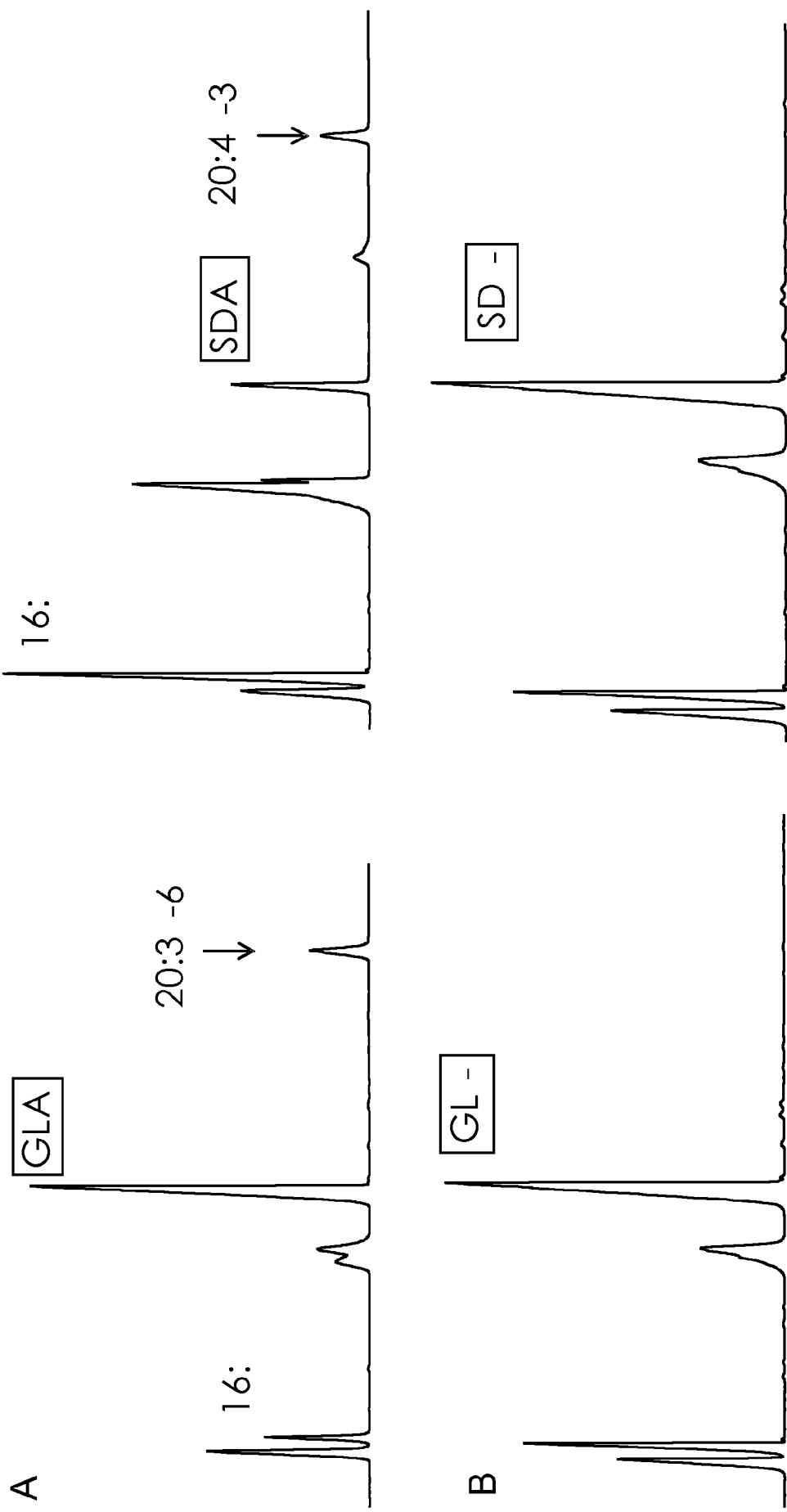


Figure 6 of 13: Gas chromatograph of yeast expression experiment with feeding of 20:4n-6 (ARA) and 20:5n-3 (EPA) in the presence (A) and absence (B) of d5Elo(Eh). The arrow indicates the formation of 22:4n-6 or 22:5n-3, respectively, the products of d5Elo(Eh) activity.

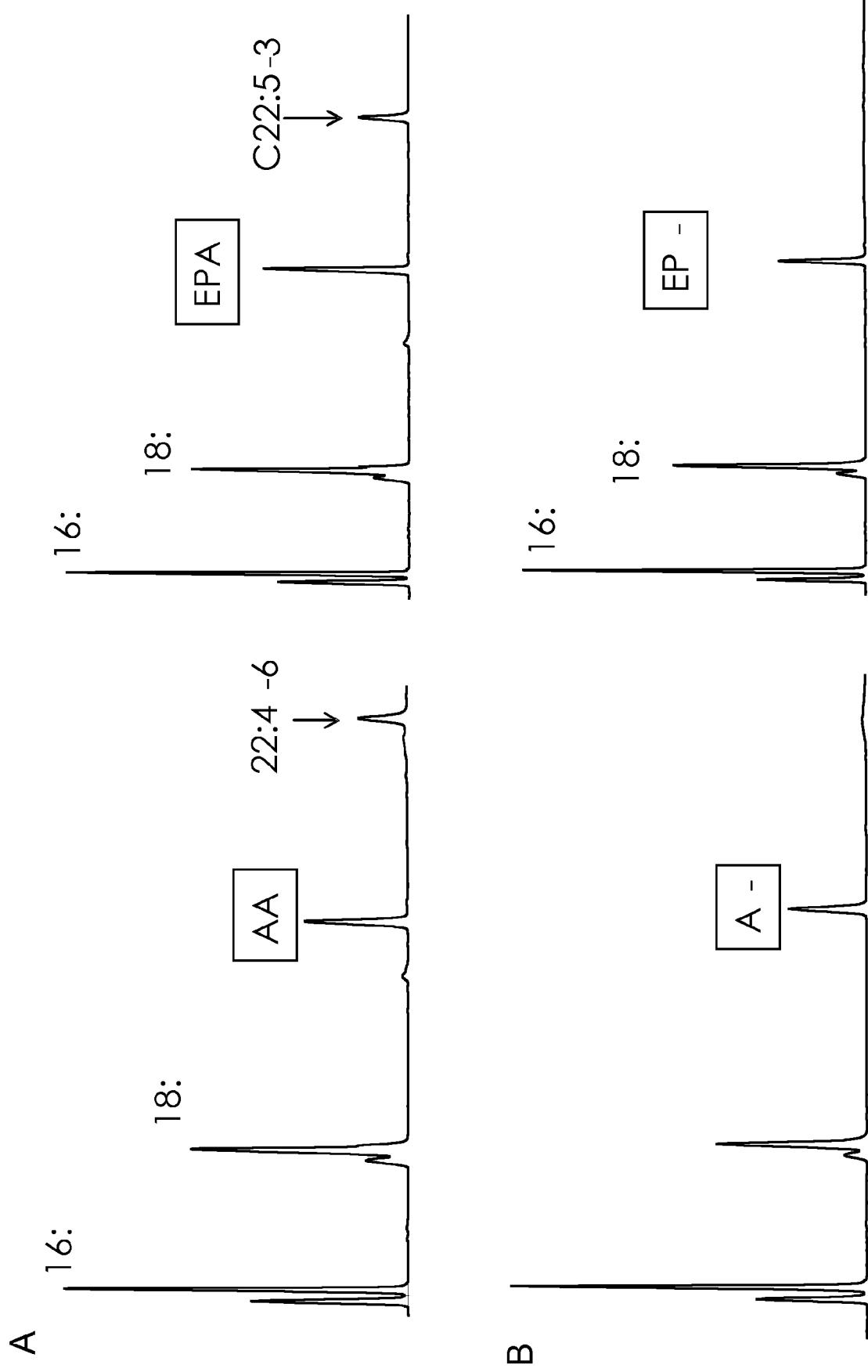


Figure 7 of 13: Gas chromatograph analysis of mature *Arabidopsis* seeds. Peaks were quantified and listed in the table below. EmiElo91 and EmiElo92 are two selected events transformed with d9Elo(Eh). WT is a non-transformed control. The products of d9Elo(Eh) activity are 20:2 and 20:3n3, which are 10fold increased compared to the levels of the non-transformed control.

Fatty	16:0	18:0	18:1	18:2	ALA	18:4	20:0	20:1	20:2	20:3n3	22:1
EmiElo9	4.64	3.43	13.34	23.88	12.64	0.18	2.55	22	11.26	3.73	1.69
EmiElo92	4.21	3.04	12.63	24.63	14.57	0.34	2.28	21.56	10.94	4.02	1.8
WT	6.22	3.21	16.17	27.75	16.63	0.16	2.4	22.2	1.95	0.46	2.15

Figure 8 of 13: Acyl-CoA analysis of mature *Arabidopsis* seeds of event EmiElo91 transformed with d9Elo(Eh). Col0 is seed material from a non-transformed plant. Δ9elo is seed material from EmiElo91. The product of d9Elo(Eh) activity is marked with a star (20:2), which is massively increased compared to the control, indicating the functional expression of d9Elo(Eh).

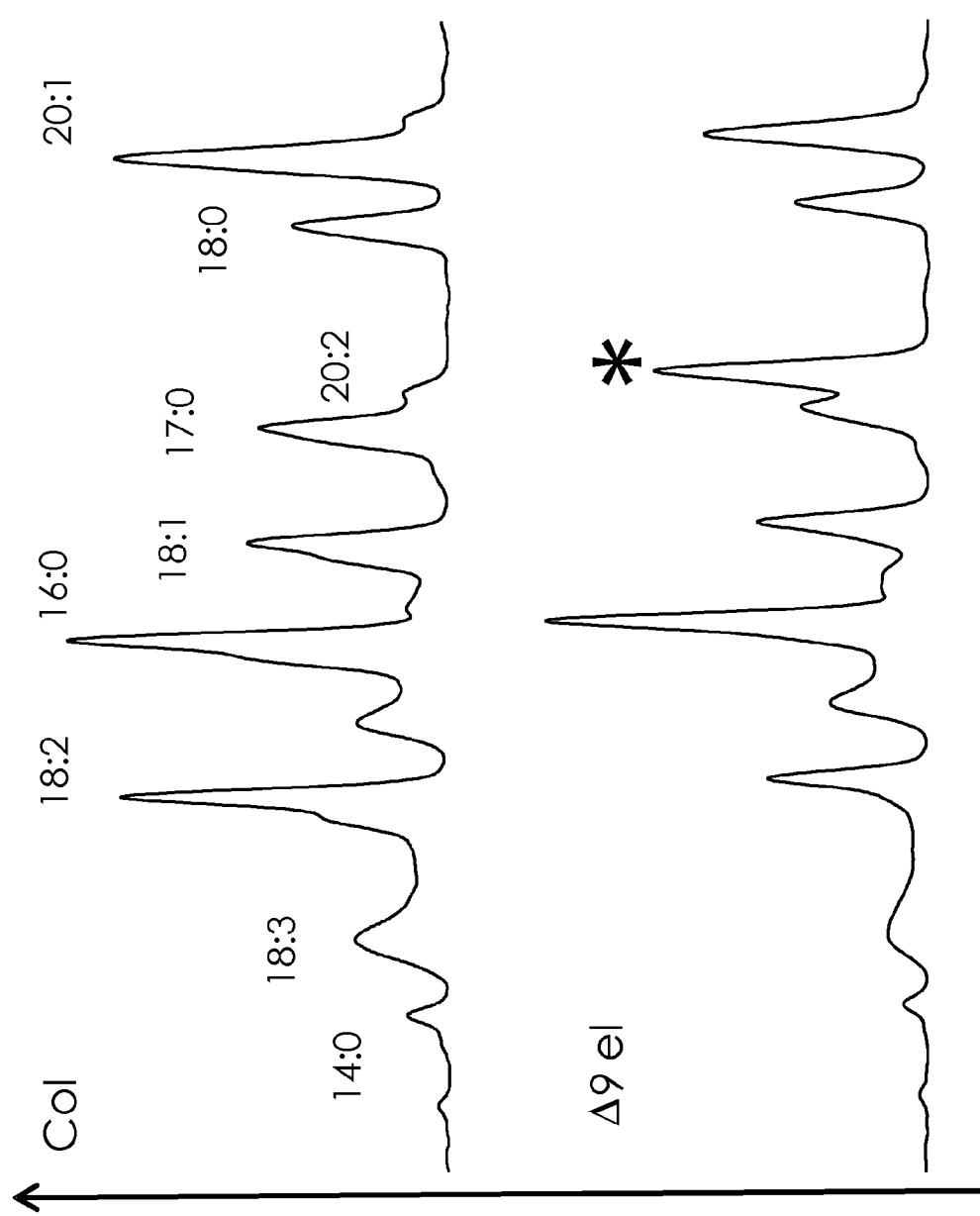


Figure 9 of 13: Gas chromatographic analysis of mature *Arabidopsis* seeds transformed with the construct AP2 (gene combinations d9Elo(Eh)_d8Des(Eh)_d5Des(Eh)_d12Des(Ps)_o3Des(Pi). Peaks were quantified and listed in the table below. The products of d9Elo(Eh) activity are 20:2 and 20:3n3.

FA	16:0	18:0	18:1c9	18:1c11	18:2	18:3n3	20:0	20:1	20:2	20:3n3
AP2 1	6.5	3.4	12.9	1.8	27.4	13.2	2.0	15.9	7.5	4.5
AP2 5	5.7	3.2	14.9	1.5	26.4	13.1	2.0	17.9	6.8	3.2
AP2 11	5.9	3.2	13.5	1.6	26.6	13.3	2.0	17.1	7.7	4.0
AP2 16	6.5	3.1	11.9	1.7	26.9	13.5	2.0	16.2	7.8	5.0
AP2 17	7.6	3.4	12.2	3.1	30.6	13.8	2.0	14.9	3.8	2.2
AP2 18	6.5	3.3	11.4	1.9	27.2	14.2	2.3	16.7	6.7	3.9
AP2 21	6.6	3.1	10.9	1.8	24.9	15.5	2.2	15.9	7.4	5.7
AP2 22	6.2	3.2	9.7	1.7	25.9	14.4	2.1	15.9	8.7	6.4
AP2 23	6.3	3.1	10.8	1.9	27.1	14.6	2.1	16.0	7.5	5.2
AP2 24	5.9	3.1	10.3	1.6	25.4	14.2	2.1	16.1	9.4	5.9
AP2 25	6.2	3.0	12.0	1.6	26.0	14.8	2.0	17.1	7.7	3.9
AP2 26	6.2	3.3	11.1	1.8	26.6	13.1	2.1	16.0	8.6	5.7
AP2 27	5.8	3.1	9.8	2.0	24.1	12.2	2.2	15.8	9.5	8.6
AP2 28	6.0	3.2	9.8	1.8	25.0	13.0	2.2	15.7	8.9	7.6
AP2 29	6.6	3.3	10.1	2.0	26.5	14.1	2.2	15.8	7.9	5.8
AP2 30	5.6	3.0	10.7	1.5	26.7	13.2	2.0	16.6	10.5	4.7
AP2 31	5.5	3.2	11.2	1.8	24.5	12.6	2.1	16.5	10.6	6.0
AP2 32	5.7	3.2	11.1	1.6	25.5	13.5	2.2	17.4	8.8	4.9
AP2 33	6.5	3.2	9.2	1.9	26.7	15.4	2.3	15.8	7.6	5.5
AP2 34	6.4	3.3	10.1	2.0	26.1	13.7	2.3	16.7	8.0	5.7
AP2 42	5.7	3.4	13.1	1.8	25.6	12.8	2.3	18.0	7.6	4.2
AP2 46	6.3	3.3	10.3	2.4	27.2	12.8	2.4	17.0	6.7	5.0
AP2 47	5.7	3.5	13.3	2.0	26.7	12.4	2.3	17.5	7.3	3.8
AP2 48	5.6	3.5	12.0	2.0	25.5	12.2	2.3	16.6	8.9	5.7
AP2 49	6.0	2.9	12.9	1.6	26.7	14.2	2.2	18.4	6.5	3.2
AP2 50	5.8	3.0	10.8	2.5	26.6	13.9	2.2	17.1	6.7	5.1
average	6.1	3.2	11.4	1.9	26.3	13.6	2.2	16.6	7.9	5.1
std	0.5	0.2	1.4	0.3	1.2	0.9	0.1	0.8	1.4	1.4

Figure 10 of 13:

FA	16:0	18:0	18:1	18:1	18:2	GLA	18:4	20:0	20:1	20:2	DHGLA	ARA	ETA	EPA	22:0	22:1	DPA	DHA
	Δ9	Δ11	Δ9,12	18:3n6				Δ11	Δ11,14	20:3n6	20:4n3	20:5n3	20:3n3	20:4n3	20:5n3	22:5	22:6	
OstELO5EmD4_1	6.	3.	10.	1.9	25.5	0.7	0.3	2.	17.9	2.0	0.	1.	2.	0.	1.	1.	2.3	
OstELO5EmD4_2	7.	3.	13.	2.4	27.4	2.7	1.5	2.	16.0	1.1	0.	0.	1.	0.	1.	0.	1.2	
OstELO5EmD4_3	6.	3.	10.	2.3	26.2	1.9	1.0	2.	15.5	1.4	0.	0.	1.	0.	3.	0.	2.7	
OstELO5EmD4_4	7.	3.	8.7	2.8	23.7	5.7	3.2	2.	15.2	1.1	0.	0.	1.	0.	2.	0.	1.6	
OstELO5EmD4_5	7.	3.	10.	2.4	24.6	3.2	1.8	2.	16.3	1.1	0.	0.	1.	2.	0.	1.	2.0	
OstELO5EmD4_6	8.	3.	9.0	2.2	23.2	6.1	4.0	2.	15.7	1.3	0.	0.	0.	1.	0.	1.	1.4	
OstELO5EmD4_7	7.	3.	7.9	2.2	24.0	1.0	0.7	2.	14.6	2.1	0.	1.	1.	1.	3.	0.	1.	
OstELO5EmD4_8	9.	3.	10.	1.7	26.8	1.1	0.5	1.	13.7	1.6	0.7	1.	0.	1.	2.	0.	1.	
OstELO5EmD4_14	8.	3.	12.	1.6	25.4	4.8	2.	2.	15.7	1.2	0.1	0.	0.	0.	1.	0.	0.	
OstELO5EmD4_15	9.	3.	11.	1.7	25.8	3.9	2.	1.	13.6	1.2	0.1	0.	0.	0.	2.	0.	0.	
OstELO5EmD4_16	7.	3.	9.7	2.2	24.3	6.7	3.	2.	14.2	0.9	0.1	0.	1.	0.	1.	0.	1.	
OstELO5EmD4_18	7.	3.	10.	2.1	24.4	5.1	3.	2.	15.7	1.3	0.5	0.	0.	0.	2.	0.	1.	
OstELO5EmD4_20	6.	3.	8.8	2.0	24.4	1.0	0.	2.	16.5	1.8	0.7	1.	1.	1.	3.	0.	1.	
OstELO5EmD4_21	5.	3.	11.	2.0	26.4	0.6	0.	2.	18.3	1.9	0.7	1.	0.	1.	2.	0.	1.	
OstELO5EmD4_25	6.	3.	10.	1.9	24.9	2.1	1.	2.	17.4	1.9	0.5	0.	0.	1.	0.	1.	1.	
OstELO5EmD4_26	6.	3.	11.	2.1	25.5	5.0	3.	1.	15.6	1.3	0.2	0.	1.	0.	1.	0.	1.	
OstELO5EmD4_27	6.	3.	12.	1.9	24.2	4.2	2.	2.	16.4	1.3	0.4	0.	0.	0.	2.	0.	1.	
OstELO5EmD4_28	8.	3.	8.2	2.4	23.5	3.7	2.	2.	14.2	1.1	0.8	1.	1.	1.	4.	0.	1.	
OstELO5EmD4_101	6.	3.	8.2	2.1	23.1	1.4	0.	3.	17.4	1.7	0.6	1.	1.	1.	3.	0.	1.	
OstELO5EmD4_103	6.	3.	10.	2.2	26.7	1.1	0.	2.	17.1	2.0	0.2	0.	1.	0.	1.	0.	1.	
OstELO5EmD4_104	6.	3.	9.2	2.4	25.3	1.2	0.	2.	15.9	1.6	0.7	1.	1.	1.	4.	0.	1.	
OstELO5EmD4_105	6.	3.	9.3	2.0	24.9	3.5	1.	2.	15.1	1.4	0.7	2.	0.	0.	3.	0.	1.	
OstELO5EmD4_106	7.	3.	7.2	2.3	19.5	3.1	2.	2.	13.8	1.3	0.4	1.	1.	1.	7.	0.	1.	
OstELO5EmD4_109	7.	3.	7.5	2.4	21.7	1.8	1.	2.	14.7	1.7	0.3	1.	1.	0.	5.	0.	1.	
OstELO5EmD4_111	7.	3.	10.	2.2	25.6	2.5	1.	2.	14.5	1.3	0.3	0.	0.	0.	3.	0.	1.	
OstELO5EmD4_112	7.	3.	6.3	3.5	20.1	2.1	1.	2.	13.3	1.7	1.0	0.	1.	2.	6.	0.	1.	
OstELO5EmD4_115	8.	3.	7.8	2.7	24.1	2.6	1.	2.	13.8	1.5	0.3	1.	1.	0.	4.	0.	1.	
OstELO5EmD4_116	7.	3.	10.	2.5	24.8	5.7	3.	2.	13.7	0.	0.2	0.	1.0	0.	2.	0.	1.	
OstELO5EmD4_117	6.	3.	9.	2.	26.	1.	0.	2.	15.2	1.	0.9	1.	1.3	1.	3.	0.	1.	
OstELO5EmD4_118	6.	3.	10.4	2.	23.	3.	2.	2.	15.3	1.	1.2	0.	1.1	2.	3.	0.	1.	
OstELO5EmD4_119	7.	3.	9.	2.	25.	4.	2.	2.	14.2	1.	0.2	0.	1.0	0.	2.	0.	0.	
OstELO5EmD4_120	6.	3.	10.0	2.	27.	1.	0.	2.	15.1	1.	0.8	1.	0.9	1.	2.	0.	1.	
average	7.	3.	9.	2.2	24.	3.0	1.	2.	15.4	1.	0.	0.	1.0	0.	3.	0.4	1.3	
std	0.	0.	1.	0.4	1.	1.8	1.	0.	1.	0.	0.	0.	0.2	0.	1.	0.1	0.2	

Figure 11 of 13: Gas chromatographic analysis of mature *Arabidopsis* seeds transformed with different constructs. Values are generated from the best individual *Arabidopsis* line. Following constructs are compared: BBC, OstELO5TcD4 and OstELO5EmD4. The construct with d4Des(Eh) delivered highest levels of DHA and highest ratio of DHA:DPA (2,9).

Line	16:	18:	18:	n-6	GL	18:	n-3	SD	20:	n-9	A	EP	DP	DH
BBC	3.9	4.1	9.9	21.0	1.0	10.5	0.6	22.	1.6	10.	-	-	-	-
OstELO TcD4	6.2	3.8	6.2	21.8	2.0	11.6	1.1	14.	1.5	5.4	4.4	4.4	4.4	3.8
OstELO EmD4	7.1	3.3	7.9	24.0	1.0	13.6	0.7	14.	1.2	3.8	1.6	1.6	1.6	4.7
BBC-OTElo5-EhD4-2	6.9	3.2	7.1	21.5	1.2	11.8	0.6	13.	1.3	4.6	8.5	8.5	8.5	0.2

Figure 12 of 13: Gas chromatographic analysis of mature *Arabidopsis* seeds transformed with the construct EmELO5Tcd4. The production of 22:5 and 22:6 demonstrate the activity of d5Elo(Eh) in seeds.

FA	16:0	18:0	18:1	18:2	GLA	ALA	18:4	20:0	20:1	20:2	DHGLA	ARA	Δ11	Δ13	Δ11,14	20:3n6	20:4n3	20:3n3	20:4n3	20:5n3	22:1	DPA	DHA
	Δ9	Δ11	Δ9,12	18:3n3																	22:5	22:6	
EmELO5Tcd4_1	6.5	3.4	10.5	2.5	24.2	3.3	12.7	2.0	2.3	17.5	3.3	1.4	0.2	0.5	1.0	0.5	4.1	1.7	0.2	0.2			
EmELO5Tcd4_2	6.4	3.6	10.0	1.9	25.2	3.1	13.1	1.8	2.3	16.1	2.4	1.4	0.2	1.0	1.2	0.5	5.6	1.3	0.2	0.2			
EmELO5Tcd4_3	6.1	3.5	14.3	1.8	27.0	0.0	16.8	0.0	2.5	18.4	2.3	3.6	0.0	0.0	1.6	0.0	0.0	1.6	0.0	0.0			
EmELO5Tcd4_4	7.0	3.6	10.1	2.4	26.2	3.4	11.9	2.1	2.3	15.9	2.9	1.1	0.3	0.6	1.2	0.5	4.3	1.2	0.3	0.4			
EmELO5Tcd4_5	6.3	3.5	8.3	2.1	23.1	1.5	12.0	0.8	2.2	15.6	2.7	1.4	1.1	1.3	1.4	2.5	8.7	1.2	0.2	0.3			
EmELO5Tcd4_6	6.1	3.6	10.6	2.1	24.9	1.6	13.3	0.8	2.3	16.6	2.5	1.8	0.9	0.7	1.3	1.7	4.8	1.3	0.2	0.2			
EmELO5Tcd4_7	5.5	3.4	10.3	1.8	25.7	0.5	12.6	0.2	2.5	18.2	2.3	2.0	0.9	1.4	1.1	1.4	6.0	1.5	0.2	0.3			
average	6.3	3.5	10.6	2.1	25.2	1.9	13.2	1.1	2.4	16.9	2.6	1.8	0.5	0.8	1.2	1.0	4.8	1.4	0.2	0.2			
std	0.5	0.1	1.8	0.3	1.3	1.4	1.7	0.9	0.1	1.1	0.4	0.9	0.4	0.5	0.2	0.9	2.6	0.2	0.1	0.1			

Figure 13 of 13: Gas chromatographic analysis of mature *Arabidopsis* seeds transformed with four different constructs (A). The production of ARA and EPA demonstrates the activity of d5Des(Eh) in seeds (B). It could be shown that d5Des(Eh) has a preference for n-6 fatty acids (ARA).

A)

	Construct							
Borage Δ6:	Borage	Glycinin promoter + Borage	Glycinin promoter + Borage	Glycinin promoter				
Ostr Δ6=		Glycinin promoter + Ostr						
Ostr Δ6:PpELO6=		Glycinin promoter + Ostr	Glycinin promoter					

B)

	16:0	18:0	18:1	18:2	18:3	18:4	18:5	18:6	20:0	20:1	20:2	20:3	20:4	20:5	20:6	EPA
Control																
Bd6+elo6	6.2	4.1	17.3	24.8	4.4	9.5	1.8	15.4	4.3	2.6	-	1.3	0.9	-		
B6/elo6/Ed5	10.8	4.3	9.3	26.0	4.4	10.7	2.0	10.6	5.0	2.0	1.4	1.7	1.0	0.5		
O6/elo6/Ed5	10.3	3.9	9.5	23.9	1.0	10.5	0.0	11.5	2.3	5.2	4.7	0.5	3.9	1.4		

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2010/060178

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/435 C12N15/82

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and where practical search terms used)

EPO-Internal , BIOSIS, Sequence Search, EMBASE, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	<p>SOTO AMELIA R ET AL: "Identification and preliminary characterization of two cDNAs encoding unique carbonic anhydrases from the marine alga <i>Emiliania huxleyi</i>." APPLIED AND ENVIRONMENTAL MICROBIOLOGY AUG 2006 LNKD- PUBMED: 16885304, vol. 72, no. 8, August 2006 (2006-08), pages 5500-5511, XP002600301 ISSN: 0099-2240 the whole document</p> <p>-----</p> <p>WO 2008/124048 A2 (DU PONT [US]; DAMUDE HOWARD G [US]; KINNEY ANTHONY J [US]; RIPP KEVIN) 16 October 2008 (2008-10-16) abstract claim all sequence 70</p> <p>-----</p> <p>-/-</p>	1
X		1-15

Further documents are listed in the continuation of Box C

See patent family annex

* Special categories of cited documents

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 September 2010	08/11/2010
Name and mailing address of the ISA/ European Patent Office, P B 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel (+31-70) 340-2040, Fax (+31-70) 340-3016	Authorized officer Keller, Yves

INTERNATIONAL SEARCH REPORTInternational application No
PCT/EP2010/060178**C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT**

Category'	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
E	WO 2009/124101 A1 (BASF PLANT SCIENCE GMBH [DE]; BAUER JOERG [DE]; NAPIER JOHNATHAN A [GB) 8 October 2010 (2010-10-08) abstract; claim all sequence 96 -----	1-15
x , P	WO 2010/042510 A1 (ABBOTT LABORATOIRES [US]; DAS TAPAS [SG]; MUKERJI PRADIP [US]; PEREIRA) 15 April 2010 (2010-04-15) the whole document -----	1-15

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/060178

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
 - a (means)
 on paper
 in electronic form
 - b (time)
 in the international application as filed
 together with the international application in electronic form
 subsequently to this Authority for the purpose of search
2. In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/060178

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons

- 1 Claims Nos because they relate to subject matter not required to be searched by this Authority, namely

- 2 Claims Nos because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically

- 3 Claims Nos because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6 4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows

see additional sheet

- 1 As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

- 2 As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees

- 3 As only some of the required additional search fees were timely paid by the applicant, this international search report covers _____ only those claims for which fees were paid, specifically claims Nos

- 4 No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims, it is covered by claims Nos

see additional sheet(s)

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation
- No protest accompanied the payment of additional search fees

FURTHER INFORMATION CONTINUED FROM POT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-15(partially)

Desaturase from Emiliana huxleyi of SEQ ID No 1, 2, 5, 6 constructs, transgenic plants containing the same and related subject matter.

2. claims: 1-15(partially)

Elongase from Emiliana huxleyi of SEQ ID No 3, 4, 7-10 constructs, transgenic plants containing the same and related subject matter.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2010/060178

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
WO 2008124048	A2 16-10-2008	AU CA CN EP JP US	2008236723 A1 2679988 A1 101765658 A 2129777 A2 2010523113 T 2008254191 A1		16-10-2008 16-10-2008 30-06-2010 09-12-2009 15-07-2010 16-10-2008
WO 2009124101	A1 08-10-2009	AU US	2009231744 A1 2009253188 A1		08-10-2009 08-10-2009
UO 2010042510	A1 15-04-2010	US	2010154080 A1		17-06-2010