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(71) Applicant *(for all designated States except US)*: **BASF PLANT SCIENCE COMPANY GMBH** [DE/DE]; 67056 Ludwigshafen (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SENGER, Toralf** [DE/DE]; Emmendinger Weg 7, 69126 Heidelberg (DE). **BAUER, Jörg** [DE/DE]; Gluckstr. 1b, 14513 Teltow (DE). **MARTY, Laurent** [CH/DE]; Albert-Fritz-Str. 31, 69124 Heidelberg (DE).

(74) Agent: **DICK, Alexander**; Herzog Fiesser & Partner, Patentanwälte, Dudenstrasse 46, 68167 Mannheim (DE).

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(54) **Title**: NOVEL FATTY ACID DESATURASES, ELONGASES, ELONGATION COMPONENTS AND USES THEROF

(57) **Abstract**: The invention provides isolated nucleic acid molecules which encodes a novel fatty acid desaturase, KCS, KCR, DH and ECR from Nannochloropsis oculata. The invention also provides recombinant expression vectors containing desaturase, KCS, KCR, DH and ECR 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.*, ARA, EPA and DHA.

NOVEL FATTY ACID DESATURASES, ELONGASES, ELONGATION COMPONENTS AND USES THEREOF

The invention in principle pertains to the field of recombinant manufacture of fatty acids. It provides nucleic acid molecules which encode desaturases, elongases and elongase components. The invention also provides recombinant expression vectors containing Desaturase, KCS, KCR, DH, ECR 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.* ARA, EPA and DHA.

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.*, b-oxidation). In addition, lipids/ fatty acids are an integral 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 *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 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 include, for example, linoleic acid (C18:2) and 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.

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 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, thrombosis and cancers (Horrocks, L.A. and Yeo, Y.K. (1999) Pharmacol. Res. 40:211-215). 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 citizerns, it is particularly important for these individuals to adequately intake these fatty acids from the diet (Spector, A.A. (1999) Lipids 34:S1-S3).

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 costs of extraction.

EPA and ARA are both delta (d) 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 Atlantic. ARA belongs to the n-6 series with four double bonds. The lack of a double bond in the ω-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 aggregating properties. ARA can be obtained from some foods such as meat, fish and eggs, but the concentration is low.

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 Δ6 desaturation. Many physiological and pathological conditions such as aging, stress, diabetes, eczema, and some infections have been shown to depress the Δ6 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 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.

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.

Thus, the present invention relates to a polynucleotide comprising a nucleic acid sequence elected from the group consisting of:

a) a nucleic acid sequence having a nucleotide sequence as shown in SEQ ID NOs: 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 46, 49, 52, 55, 58, 61 or 128

b) a nucleic acid sequence encoding a polypeptide having an amino acid sequence as shown in SEQ ID NOs: 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62 or 129

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, keto-acyl-CoA synthase (KCS), keto-acyl-CoA reductase (KCR), dehydratase (DH) or enoyl-CoA reductase (ECR) activity;

d) a nucleic acid sequence encoding a polypeptide having desaturase, keto-acyl-CoA synthase (KCS), keto-acyl-CoA reductase (KCR), dehydratase (DH) or enoyl-CoA reductase (ECR) activity and having an amino acid sequence which is at least 70% 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, keto-acyl-CoA synthase (KCS), keto-acyl-CoA reductase (KCR), dehydratase (DH) or enoyl-CoA reductase (ECR) activity.

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, keto-acyl-CoA-synthase, keto-acyl-CoA-reductase, dehydratase and enoyl-CoA-reductase activity. Preferably, the polypeptide encoded by the polynucleotide of the present invention having desaturase, KCS, KCR, DH and ECR activity upon expression in a plant 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 minimal set of desaturases and elongases

requiered for LCPUFA synthesis but does not express the polynucleotide of the present invention. 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 said control. Preferably, the LCPUFA referred to before is a polyunsaturated fatty acid having a C-20, C-22 or C-24 fatty acid body, more preferably, ARA, EPA or DHA. Suitable assays for measuring the activities mentioned before are described in the accompanying Examples.

The term " desaturase" encompasses all enymatic activities and enzymes catalyzing the desaturation of fatty acids with different lengths and numbers of unsaturated carbon atom double bonds. Specifically this includes delta 4 (d4)-desaturase, catalyzing the dehydrogenation of the 4^{th} and 5^{th} carbon atom. Delta 5 (d5)-desaturase catalyzing the dehydrogenation of the 5^{th} and 6^{th} carbon atom. Delta 6 (d6)-desaturase catalyzing the dehydrogenation of the 6^{th} and 7^{th} carbon atom. Delta 8 (d8)-desaturase catalyzing the dehydrogenation of the 8^{th} and 9^{th} carbon atom. Delta 9 (d9)-desaturase catalyzing the dehydrogenation of the 9^{th} and 10^{th} carbon atom. Delta 12 (d12)-desaturase catalyzing the dehydrogenation of the 12^{th} and 13^{th} carbon atom. Delta 15 (d15)-desaturase catalyzing the dehydrogenation of the 15^{th} and 16^{th} carbon atom.

The terms " elongase" and " delta x Elo (dxElo)" are synonymous to KCS and refer to keto-acyl-CoA-synthase enzymatic activity, which allows to introduce two carbon atoms in a fatty acid whereby the fatty acid is elongated. Specifically, dxElo(No) catalyzes the introduction of two carbon atoms into fatty acids having 18 carbon atoms and double bonds in the positions 5, 6, 9, 12 and/or 15, respectively.

The term " KCR" as used herein refer to keto-acyl-CoA-reductase activity, which reduces the keto-group of keto-acyl-CoA to a hydroxyl-group, in the process of fatty acid elongation.

The term " DH" as used herein refers to dehydratase activity, removing the hydroxyl-group leading to the formation of a acyl-2-en-CoA ester (delta-2-enoyl-CoA) and H_2O during fatty acid elongation.

The term " ECR" as used herein refers to enoyl-CoA reductase activity, reducing the double bond of delta-2-enoyl-CoA, in course of fatty acid elongation, generating the elongated acyl-CoA ester.

Fatty acid elongation is catalyzed in four steps, represented by four enzymes: KCS (keto-acyl-CoA-synthase), KCR (keto-acyl-CoA-reductase), DH (dehydratase) and ECR (enoyl-CoA-reductase). In the first step a fatty acid-CoA ester is condensed with malonyl-

CoA producing a keto-acly-CoA intermediate, which is elongated by two carbon atoms, and CO_2. The keto-group of the intermediate is then reduced by the KCR to a hydroxyl-group. In the next step the DH cleaves of the hydroxyl-group (H_2O is produced), forming a acyl-2-en-CoA ester (delta-2-enoyl-CoA). In the final step the double bound at position 2, 3 is reduced by the ECR forming the elongated acyl-CoA ester (Buchanan, Gruissem, Jones (2000) Biochemistry & Molecular biology of plants, American Society of Plant Physiologists).

In the studies underlying this invention, enzymes with superior desaturase, KCS, KCR, DH, and ECR catalytic activities for the production of PUFA has been provided.

More preferably, polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 1 encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 2 or variants thereof, preferably, exhibit d5-desaturase activity.

Polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 4 encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 5 or variants thereof, preferably, exhibit d6-desaturase activity.

Polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 7 and 128 encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 8 and 129 or variants thereof, preferably, exhibit d4-desaturase activity.

Polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 10 encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 11 or variants thereof, preferably, exhibit d8-desaturase activity.

Polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 13 encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 14 or variants thereof, preferably, exhibit d9-desaturase activity.

Polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 16 encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 17 or variants thereof, preferably, exhibit d12-desaturase activity.

Polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 19 encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 20 or variants thereof, preferably, exhibit d15-desaturase activity.

Polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 22, 25, 28, 31, 34, 37, 40, 43 or 46 encoding polypeptides having amino acid sequences as shown

in SEQ ID NOs: 23, 26, 29, 32, 35, 38, 41, 44 or 46 or variants thereof, preferably, exhibit keto-acyl-CoA synthase activity.

Polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 49, 52 or 55 encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 50, 53 or 56 or variants thereof, preferably, exhibit keto-acyl-CoA reductase activity.

Polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 58 encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 59 or variants thereof, preferably, exhibit dehydratase activity.

Polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 61 encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 62 or variants thereof, preferably, exhibit enoyl-CoA-reductase activity.

A polynucleotide encoding a polypeptide having a desaturase, KCS, KCR, DH and ECR activity as specified above has been obtained in accordance with the present invention, preferably, from Nannochloropsis oculata. However, orthologs, paralogs or other homologs may be identified from other species. Preferably, they are obtained from plants such as algae, for example Isochrysis, Mantoniella, Ostreococcus or Crypthecodinium, 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 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 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 Thalassiosira 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, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 46, 49, 52, 55, 58, 61 or 128 by a polynucleotide encoding a polypeptide having an amino acid sequence as shown in any one of SEQ ID NOs: 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62 or 129 by at least one nucleotide substitution, addition and/or deletion, whereby the variant nucleic acid sequence shall still encode a polypeptide having a desaturase, KCS, KCR, DH and ECR 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 × sodium chloride/sodium citrate (= SSC) at approximately 45°C, followed by one or more wash steps in 0.2 × 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 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 × 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 × SSC and 20°C to 45°C, preferably between 30°C and 45°C. The hybridization conditions for DNA:RNA hybrids are, preferably, 0.1 × 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 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, 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 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 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, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 46, 49, 52, 55, 58, 61 or 128 preferably, encoding polypeptides retaining desaturase, KCS, KCR, DH and ECR 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 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, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62 or 129 wherein the polypeptide, preferably, retains desaturase, KCS, KCR, DH and ECR 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 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,I., 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 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 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,I., and Bleasby,A, Trends in Genetics 16(6), *276-277, 2000)*, using 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 nucleic 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 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 desaturase, KCS, KCR, DH and ECR nucleic acid sequences of the invention as query sequence can be 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 desaturase, KCS, KCR, DH and ECR sequences of the invention. BLAST using desaturase, KCS, KCR, DH and ECR 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 (BLASTp) or nucleic acid sequences (tBLASTn) homologous to desaturase, KCS, KCR, DH and ECR 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).

Table 1: Relation of sequence types: DNA or PRT (Protein) of query- and hit-sequences for various BLAST programs

Input sequence	query 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 fragments shall encode polypeptides which still have desaturase, KCS, KCR, DH or ECR 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, KCS, KCR, DH or ECR 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, KCS, KCR, DH or ECR activity exhibited by any of the polypeptide shown in any one of SEQ ID NOs: 2, 5,

8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62 and 129. 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 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.

However, the present invention also pertains to polynucleotide variants which are derived from the polynucleotides of the present invention and are capable of interefering 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, 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.

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.

In the studies underlying the present invention, advantageously, polynucleotides where identified encoding desaturases, keto-acyl-CoA-synthases, keto-acyl-CoA-reductases, dehydratases and enoyl-CoA-reductases from *Nannochloropsis oculata* or *Monosiga brevicollis*. In particular, the *Nannochloropsis oculata* d4-desaturase (d4Des(No)), d5-desaturase (d5Des(No)), d6-desaturase (d6Des(No)), d8-desaturase (d8Des(No)), d9-desaturase (d9Des(No)), d12-desaturase (d12Des(No)), d15-desaturase (d15Des(No)) keto-acyl-CoA-synthase (Elo(No)), keto-acyl-CoA-reductase (KCR(No)), dehydratase (DH(No)) and enoyl-CoA-reductase (ECR(No)) have been identified. In addition, in particular, the Monosiga brevicollis d4-desautrase d4Des(Mb) has been identified. The polynucleotides of the present invention are particularly suitable for the recombinant manufacture of LCPUFAs and, in particular, arachidonic acid (ARA), eicosapentaenoic acid (EPA) and/or docosapentaenoic acid (DHA).

In a preferred embodiment of the polynucleotide of the present invention, said 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 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, AOX1r, GAL1, MFα , 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 A1 (i.e. a benzylsulfonamide-inducible promoter), Gatz 1992, Plant J. 2:397-404 (i.e. a tetracyclin-inducible promoter), EP 0 335 528 A1 (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 A1. 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: lpt-2 or lpt-1 promoter from barley (WO 95/15389 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.

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 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 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.

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 homolgous or heterologous recombination as described in 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 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 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 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.

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, 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 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, 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, 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 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 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), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (Invitrogene) or pSPORT1 (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-III113-B1, λgt11 or pBdCl, in Streptomyces pIJ101, pIJ364, pIJ702 or pIJ361, in Bacillus pUB110, 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 54:113-123) 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. 20:1195-1197; Bevan 1984, Nucl. Acids Res. 12:8711-8721; 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 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 sequence, which comprises the 5' -untranslated tobacco mosaic virus leader sequence, which increases the protein/RNA ratio (Gallie 1987, Nucl. Acids Research 15:8693-8711). 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, 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 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 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. 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 hsp80 promoter from tomato (US 5,187,267), the cold-inducible alpha-amylase promoter from potato (WO 96/12814) or the wound-inducible pinII promoter (EP 0 375 091 A). The promoters which are especially preferred are those which 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 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 lpt2 or lpt1 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 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 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, Amsterdam-New York-Oxford, 1985, ISBN 0 444 904018). 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 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.

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 oilseed crop. More preferably, said oilseed crop is selected from the group consisting of flax (Linum sp.), rapeseed (Brassica sp.), soybean (Glycine and Soja sp.), sunflower (Helianthus sp.), cotton (Gossypium sp.), corn (Zea mays), olive (Olea sp.), safflower (Carthamus sp.), cocoa (Theobroma cacoa), peanut (Arachis sp.), hemp, camelina, crambe, oil palm, coconuts, groundnuts, sesame seed, castor bean, lesquerella, tallow tree, sheanuts, tungnuts, kapok 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.

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 unsaturated fatty acid biosynthetic pathway, are catalyzed by membrane-associated fatty acid elongase complexes. Plants and most other eukaryotic organisms have specialized elongase system for the extension of fatty acids beyond C18 atoms. These elongase reactions have several important features in common with the fatty acid synthase complex (FAS). However, the elongase 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-CoA-synthase (KCS, condensation reaction of malonyl-CoA to acyl-CoA, creation of a 2 C atom longer keto-acyl-CoA fatty acid), the keto-acyl-CoA-reductase (KCR, reduction of the 3-keto group to a 3-hydroxy-group), the dehydratase (DH, dehydration results in a delta-2-enoyl-acyl-CoA fatty acid) and the enoly-CoA-reductase (ECR, reduction of the double bond at position 2, release from the complex). For the production of LCPUFAs including ARA, EPA and/or DHA the elongation and desaturation reactions could be essential. Higher plants do not have the 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. Critical steps in the process of LCPUFA biosynthesis are the elongation of fatty acids from 18 to 24 carbon atoms and desaturation of carbon atoms. Polynucleotides of the present invention surprisingly catalyze the keto-acyl-CoA-synthase, keto-acyl-CoA-reductase, dehydratase, enoyl-CoA-reductase reactions and therefore catalyze the elongation of 18 carbon atoms fatty acids. Polynucleotides of the present invention surprisingly catalyze the desaturation of the 4^{th}, 5^{th}, 8^{th}, 9^{th}, 12^{th} and 15^{th} fatty acids carbon atom bonds. By delivering these enzymes increased levels of PUFAs and LCPUFAs are produced.

However, it will be understood 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 desaturases and/or elongases which shall be present in the host cell are at least one

enzyme selected from the group consisting of: d4-desaturase, d5-desaturase, d5-elongase, d6-desaturase, d12-desaturase, d15-desaturase, ω3-desaturase d-6-elongase or d-9-elongase. Especially prefered are the bifunctional d12d15-desaturases d12d15Des(Ac) from Acanthamoeba castellanii (WO2007042510), d12d15Des(Cp) from Claviceps purpurea (WO2008006202) and d12d15Des(Lg)1 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), d12Des(Nh) from Nectria haematococca (WO2009016202), d12Des(Ol) 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 (WO2009016202), d15Des(Mc) from Microcoleus chthonoplastes (WO2009016202), d15Des(Mf) from Mycosphaerella fijiensis (WO2009016202), d15Des(Mg) from Mycosphaerella graminicola (WO2009016202) and 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(Ol)2 from Ostreococcus lucimarinus (WO2008040787), d5Des(Pp) from Physcomitrella patens (WO2004057001), d5Des(Pt) from Phaeodactylum tricornutum (WO2002057465), d5Des(Tc) from Thraustochytrium sp. (WO2002026946), d5Des(Tp) from Thalassiosira pseudonana (WO2006069710) and the d6-desaturases d6Des(Cp) from Ceratodon purpureus (WO2000075341), d6Des(Ol) from Ostreococcus lucimarinus (WO2008040787), d6Des(Ot) from Ostreococcus tauri (WO2006069710), d6Des(Pf) from Primula farinosa (WO2003072784), d6Des(Pir)_BO from Pythium irregulare (WO2002026946), d6Des(Pir) from Pythium irregulare (WO2002026946), d6Des(Plu) from Primula luteola (WO2003072784), d6Des(Pp) from Physcomitrella patens (WO200102591), 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 (EP1790731), d8Des(Eg) from Euglena gracilis (WO200034439) and d8Des(Pm) from Perkinsus marinus (WO2007093776), the o3-desaturases o3Des(Pi) from Phytophthora infestans (WO2005083053), o3Des(Pir) from Pythium irregulare (WO2008022963), o3Des(Pir)2 from Pythium irregulare (WO2008022963) and o3Des(Ps) from Phytophthora sojae (WO2006100241), the bifunctional d5d6-elongases d5d6Elo(Om)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(Ol) from Ostreococcus lucimarinus

(WO2008040787), d5Elo(Ot) from Ostreococcus tauri (WO2005012316), d5Elo(Tp) from Thalassiosira pseudonana (WO2005012316) and d5Elo(Xl) from Xenopus laevis (WO2005012316), the d6-elongases d6Elo(Ol) from Ostreococcus lucimarinus (WO2008040787), d6Elo(Ot) from Ostreococcus tauri (WO2005012316), d6Elo(Pi) from Phytophthora infestans (WO2003064638), d6Elo(Pir) from Pythium irregulare (WO2009016208), d6Elo(Pp) from Physcomitrella patens (WO2001059128), d6Elo(Ps) from Phytophthora sojae (WO2006100241), d6Elo(Ps)2 from Phytophthora sojae (WO2006100241), d6Elo(Ps)3 from Phytophthora sojae (WO2006100241), d6Elo(Pt) from Phaeodactylum tricornutum (WO2005012316), d6Elo(Tc) from Thraustochytrium sp. (WO2005012316) and d6Elo(Tp) from Thalassiosira pseudonana (WO2005012316), the d9-elongases d9Elo(Ig) from Isochrysis galbana (WO2002077213), d9Elo(Pm) from Perkinsus marinus (WO2007093776) and d9Elo(Ro) from Rhizopus oryzae (WO2009016208). Particularly, if the manufacture of ARA is envisaged in higher plants, the enzymes recited in table 5 or 6, below (i.e. additionally a d6-desaturase, d6-elongase, d5-desaturase, and d12-desaturase) 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 7, below (i.e. additionally a d6-desaturase, d6-elongase, d5-desaturase, d12-desaturase, omega 3-desaturase and d15-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 8, below (i.e. aditinonally a d6-desaturase, d6-elongase, d5-desaturase, d12-desaturase, omega 3-desaturase, d15-desaturase, d5-elongase, and d4-desaturase), or enzymes having essentially the same activity may be combined in a host cell.

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 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

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 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 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.

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

The term " polypeptide" as used herein encompasses essentially purified polypeptides or 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 posttranslational modifications are known (see full ABFRC Delta mass list at http://www.abrf.org/index.cfm/dm.home). The polypeptide of the present invention shall exhibit the desaturase, keto-acyl-CoA-synthase, keto-acyl-CoA-reductase, dehydratase and enoyl-CoA-reductase activity referred to above.

Encompassed by the present invention is, furthermore, an antibody 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 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 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 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 Galfré 1981, Meth. Enzymol. 73, 3, which comprise the fusion of mouse myeloma cells to spleen cells derived from immunized mammals. The antibodies can be 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.

Moreover, the antibody according to the present invention can be applied for identifying the presence or absence of the polypeptides of the present invention. Preferably, the antibody is used for identifying non-human transgenic organisms as specified elsewhere herein and, preferably, transgenic plants, which comprise the polypeptides of the present invention. To this end, the antibody may be provided in form of a kit which allows for identifying non-human transgenic organisms and, preferably, transgenic plants comprising the polypeptides of the present invention. The kit, in addition to the antibody of the present invention, may further comprise a detection agent for detecting a complex of the antibody of the invention and the polypeptide of the invention.

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. 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, Cryptheccodiniaceae, 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], 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 or french 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], Boraginaceae, such as the genus Borago, for example the genus and species Borago officinalis [borage], Brassicaceae, such as the genera Brassica, Melanosinapis, Sinapis, Arabadopsis, 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], 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 batatus, 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], Crypthecodiniaceae, such as the genus Crypthecodinium, 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, 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 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, Distichium macounii, Eccremidium floridanum, Eccremidium whiteleggei, Lophidion strictus, Pleuridium acuminatum, Pleuridium alternifolium, 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], 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, Pithecolobium, Acacia, Mimosa, Medicajo, 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 berteriana, Albizzia berteriana, Cathormion berteriana, Feuillea berteriana, Inga fragrans, Pithecellobium berterianum, Pithecellobium fragrans, Pithecolobium berterianum, Pseudalbizzia berteriana, Acacia julibrissin, Acacia nemu, Albizia nemu, Feuilleea julibrissin, Mimosa julibrissin, Mimosa speciosa, Sericanrda julibrissin, Acacia lebbeck, Acacia macrophylla, Albizia lebbek, Feuilleea lebbeck, Mimosa lebbeck, Mimosa speciosa [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 Aphanorrhegma, Entosthodon, Funaria, Physcomitrella, Physcomitrium, for example the genera and species Aphanorrhegma serratum, Entosthodon attenuatus, Entosthodon bolanderi, Entosthodon bonplandii, 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. 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, 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 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, Wallia, for example the genera and species Juglans regia, Juglans ailanthifolia, Juglans sieboldiana, Juglans cinerea, Wallia cinerea, Juglans bixbyi, 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, 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 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 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 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, 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, 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, Sorghum miliaceum, Panicum militaceum [millet], Oryza sativa, Oryza latifolia [rice], Zea mays [maize], Triticum aestivum, Triticum durum, Triticum turgidum, Triticum hybernum, 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, 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, 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, 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, Tagetes, Solanaceae plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa or bushy plants (coffee, cacao, tea), Salix 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 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.

Preferred mosses are Physcomitrella or Ceratodon. Preferred algae are Isochrysis, Mantoniella, Ostreococcus or Crypthecodinium, and algae/diatoms such as Phaeodactylum or Thraustochytrium. More preferably, said algae or mosses are selected from the group consisting of: Shewanella, Physcomitrella, Thraustochytrium, Fusarium, Phytophthora, Ceratodon, Isochrysis, Aleurita, Muscarioides, Mortierella, Phaeodactylum, Cryphthecodinium, specifically from the genera and species Thalassiosira pseudonona, Euglena gracilis, Physcomitrella patens, Phytophthora infestans, Fusarium graminaeum, Cryptocodinium cohnii, Ceratodon purpureus, Isochrysis galbana, Aleurita farinosa, Thraustochytrium sp., Muscarioides viallii, Mortierella alpina, Phaeodactylum tricornutum or Caenorhabditis elegans or especially advantageously Phytophthora infestans, Thalassiosira pseudonona and Cryptocodinium 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 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 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 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 elsewhere in this specification (see above and tables 5, 6 and 7).

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 of polyunsaturated fatty acids in said host cell; and
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 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 (8,11,14), ARA 20:4 (5,8,11,14), iARA 20:4(8,11,14,17), EPA 20:5 (5,8,11,14,17), DPA 22:5 (4,7,10,13,16), DHA 22:6 (4,7,10,13,16,19), 20:4 (8,11,14,17), more preferably, arachidonic acid (ARA) 20:4 (5,8,11,14), eicosapentaenoic acid (EPA) 20:5 (5,8,11,14,17), and docosahexaenoic acid (DHA) 22:6 (4,7,10,13,16,19). Thus, it will be understood that most 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, keto-acyl-CoA-synthase, keto-acyl-CoA-reductase, dehydratase and enoyl-CoA-reductase activity of the polypeptide of the present invention. Preferably, substrates encompass LA 18:2 (9,12), GLA 18:3 (6,9,12), DGLA 20:3 (8,11,14), ARA 20:4 (5,8,11,14), eicosadienoic acid 20:2 (11,14), eicosatetraenoic acid 20:4 (8,11,14,17), eicosapentaenoic acid 20:5 (5,8,11,14,17).

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 present invention is expressed in the host cell so that the desaturase, keto-acyl-CoA-synthase, keto-acyl-CoA-reductase, dehydratase and enoyl-

CoA-reductase 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 estres. 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.

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 depedent 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 fermentation or administerd 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 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 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 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, 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 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, 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.

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 host cell; 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.

For the production of ARA it is, preferably, envisaged to cultivate a host cell of the invention or a non-human transgenic organism which comprises a combination of polynucleotides of the present invention. Preferably, a combination of the polynucleotides of the invention is envisaged which encode a d12 desaturase, a d6 desaturase, a d6

elongase, a d5 desaturase KCR, DH and ECR (see also Table 6 in the accompanying Examples).

For the production of ARA it is, alternatively but also preferably, envisaged to cultivate a host cell of the invention or a non-human transgenic organism which comprises a combination of polynucleotides of the present invention. Preferably, a combination of the polynucleotides of the invention is envisaged which encode a d12 desaturase, a d9 elongase, a d8 desaturase, a d6 elongase, a d5 desaturase KCR, DH and ECR (see also Table 7 in the accompanying Examples).

For the production of EPA it is, preferably, envisaged to cultivate a host cell of the invention or a non-human transgenic organism which comprises a combination of polynucleotides of the present invention. Preferably, a combination of the polynucleotides which are preferably applied for the ARA production specified above is used together with a polynucleotide of the present invention encoding a d15 desaturase and a polynucleotide of the present invention encoding a omega-3 desaturase (i.e. a combination of the activities referred to either in Table 6 with those of Table 8 or Table 7 with those of Table 8; see also Table 8 in the accompanying Examples).

For the production of DHA it is, preferably, envisaged to cultivate a host cell of the invention or a non-human transgenic organism which comprises a combination of polynucleotides of the present invention. Preferably, a combination of the polynucleotides which are preferably applied for the EPA production specified above is used together with a polynucleotide of the present invention encoding a d5 elongase and a polynucleotide of the present invention encoding a d4 desaturase (i.e. a combination of the activities referred to either in Table 6 and Table 8 with those of Table 9 or Table 7 and Table 8 with those of Table 9; see also Table 9 in the accompanying Examples).

The present invention also relates to an oil comprising a polyunsaturated fatty acid obtainable by the aforementioned methods.

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 is known that most of the fatty acids in plant oil are esterified in triacylglycerides. Accordingly, in the oil of the invention, the PUFAs and LCPUFAs are, preferably, also occur in esterified form in the triacylglcerides. 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.

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 specification.

FIGURES

The Figure shows the production of d4/d5/d6/d15 desaturated fatty acids in yeast transformed with pYes-pd4Des(Mb), pYes-pd5Des_c738(No) or pYes-pd6Des_c2410(No) construct. The fatty acid spectrum of transgenic yeast fed with different fatty acid are depicted. A: control pYes fed with 22:4n-6, B: pYes fed with 22:5n-3, C: pYes-pd4Des(Mb) fed with 22:4n-6, D: pYes-pd4Des(Mb) fed with 22:5n-3, E: pYes control fed with 20:3n-6, F: pYes control fed with 20:4n-3, G: pYes-pd5Des_c738(No) fed with 20:3n-6, H: pd5Des_c738(No) fed with 20:4n-3, I: control pYes fed with 18:2n-6, J: pYes control fed with 18:3n-3, K: pYes-pd6Des_c2410(No) fed with 18:2n-6 and L: pYes-pd6Des_c2410(No) fed with 18:3n-3.

The invention will now be illustrated by the following Examples which, however, shall not be construed as limiting the scope of the invention.

EXAMPLES

Example 1: General Cloning Methods

Cloning methods as e.g. use of restriction endonucleases to cut double stranded DNA at specific sites, agarose gel electrophoreses, purification of DNA fragments, transfer of nucleic acids onto nitrocellulose and nylon membranes, ligation of DNA fragments,

transformation of E.coli cells and culture of bacteria were performed as described in Sambrook et al. (1989) (Cold Spring Harbor Laboratory Press: ISBN 0-87965-309-6).

Example 2: Sequence Analysis of recombinant DNA

Sequencing of recombinant DNA molecules was performed using a laser-fluorescence DNA sequencer (Applied Biosystems Inc, USA) employing the sanger method (Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467). Expression constructs harboring fragments obtained by polymerase chain reaction were subjected to sequencing to confirm the correctness of the expression cassettes consisting of promoter, nucleic acid molecule to be expressed and terminator to avoid mutations that might result from handling of the DNA during cloning, e.g. due to incorrect primers, mutations from exposure to UV-light or errors of polymerases.

Example 3: Cloning of yeast expression construct via homologous recombination

The open reading frame listed in SEQ ID NOs: 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, 52, 55, 58, 61 and 128 encoding polypeptides with the amino acid sequence SEQ ID NOs: 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62 and 129 that have desaturase, elongase, KCR, DH and ECR activity can be amplified using the primers listed in table 2 in a polymerase chain reaction. By doing so, the open reading frame is 5' fused to about 60 nucleotides of the 3' end of the GAL1 promoter sequence with simultanious introduction of an *Asc* I and/or *Nco* I restriction site between the fusion site and 3' fused to about 60 nucleotides of the 5' end of the CYC1 terminator sequence with simultanious introduction of an *Pac* I restriction site. To integrate these fragments into pYES2.1 TOPO downstream of the galactose inducible GAL1 Promoter via homologous recombination, the vector pYES2.1 (Invitrogen) can be digested using the restriction endonucleases *Pvu* II and *Xba* I, and Saccharomyces cerevisiae can be transformed with 5 to 20 ng of linearized pYES2.1 TOPO vector and 20 to 100 ng PCR product per 50 µl competent cells using the transformation method described by Schiestl et al. (Schiestl et al. (1989) Curr. Genet. 16(5-6), pp. 339-346), to obtain pYes-pd5Des_c738(No), pYes-pd6Des_c2410(No), pYes-pd4Des_c5834(No), pYes-pd8Des_c20493(No), pYes-pd9Des_c3000(No), pYes-pd12Des_c6209(No), pYes-pd15Des_c3421(No), pYes-pdxElo_c1013(No), pYes-pdxElo_c10303(No), pYes-pdxElo_c2186(No), pYes-pdxElo_c2529(No), pYes-pdxElo_c37(No), pYes-pdxElo_c38(No), pYes-pdxElo_c4958(No), pYes-pdxElo_c21679(No), pYes-pdxElo_lrc26016(No), pYes-pKCR_c20574(No), pYes-pKCR_c20772(No), pYes-pKCR_c2845(No), pYes-pDH_c7190(No), pYes-pECR_c41(No) and pYes-pd4Des(Mb) in various wildtype yeasts. Positive transformants can be selected based on the complementation of the URA auxotrophy of the chosen S. cerevisiae strain. To validate the correctness of the expression construct harbored by a particular yeast clone,

plasmids can be isolated as described in Current Protocols in Molecular Biology (Hoffmann, Curr. Protoc. Mol. Biol. 2001 May; Chapter 13:Unit13.11), transformed into E. coli for amplification and subjected to sequencing of the expression cassette as described in Example 2.

Table 2: Primer sequences for cloning polynucleotides of desaturase, keto-acyl-CoA-synthase, keto-acyl-CoA-reductase, dehydratase and enoyl-CoA-reductase of the invention for expression in yeast

Gene-Name	Primer	SEQ-ID
pd5Des_c738(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtcaaggagaaaaaaccccggatcggcgcgccaccatgccgccccagaacgacgccgc	64
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactcctttcctttttcggttagagcggatttaattaactagcccatgtgcacctccgccg	65
pd6Des_c2410(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtcaaggagaaaaaaccccggatcggcgcgccaccatgggacgcggtggcgagcggat	66
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactcctttcctttttcggttagagcggatttaattaattacatggcggggaagtcggcca	67
pd4Des_c5834(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtcaaggagaaaaaaccccggatcggcgcgccaccatggccgatgtcgagtccatcaa	68
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactcctttcctttttcggttagagcggatttaattaattacgaagaggaggttatgttgg	69
pd8Des_c20493(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtcaaggagaaaaaaccccggatcggcgcgccaccatggcgccgcgcgatgtggagac	70
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactcct	71

	tccttttcggttagagcggatttaattaattaccccgccgccgccgttgttg	
	Forward:	
	ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca	
	aggagaaaaaaccccggatcggcgcgccaccatggtcttccagctc	
pd9Des_c3000(No)	gcccgaga	72
	Reverse:	
	aactataaaaaaataaatagggacctagacttcaggttgtctaactcct	
	tccttttcggttagagcggatttaattaattaattgtacttggggtgattac	73
	Forward:	
	ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca	
pd12Des_c6209(No	aggagaaaaaaccccggatcggcgcgccaccatgggacgcggcg	
)	gtgagaagac	74
	Reverse:	
	aactataaaaaaataaatagggacctagacttcaggttgtctaactcct	
	tccttttcggttagagcggatttaattaactatgctcgctgcttgtagaaca	75
	Forward:	
	ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca	
pd15Des_c3421(No	aggagaaaaaaccccggatcggcgcgccaccatggttgagcaaac	
)	attgccgac	76
	Reverse:	
	aactataaaaaaataaatagggacctagacttcaggttgtctaactcct	
	tccttttcggttagagcggatttaattaattacggaggggaggaagaac	
	ggg	77
	Forward:	
	ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca	
	aggagaaaaaaccccggatcggcgcgccaccatgaagtgggtcct	
pdxElo_c1013(No)	gcaagaagg	78
	Reverse:	
	aactataaaaaaataaatagggacctagacttcaggttgtctaactcct	
	tccttttcggttagagcggatttaattaactactgtgcttttgtcttaccct	79
	Forward:	
	ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca	
	aggagaaaaaaccccggatcggcgcgccaccatgtcttggtttttgga	
pdxElo_c10303(No)	ccccgc	80
	Reverse:	
	aactataaaaaaataaatagggacctagacttcaggttgtctaactcct	
	tccttttcggttagagcggatttaattaattacgccatcttctttccattcc	81
	Forward:	
	ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca	
	aggagaaaaaaccccggatcggcgcgccaccatgctgagcaaaa	
pdxElo_c2186(No)	gcttcaatac	82

	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactcct tccttttcggttagagcggatttaattaactactgtgctttcttcaagtcca	83
pdxElo_c2529(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca aggagaaaaaaccccggatcggcgcgccaccatggaggccccct cccgcacct	84
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactcct tccttttcggttagagcggatttaattaatcacctttctggggaggcaccc g	85
pdxElo_c37(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca aggagaaaaaaccccggatcggcgcgccaccatggccgccgccct tctttcaga	86
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactcct tccttttcggttagagcggatttaattaattaaatcttcttgagagccggct	87
pdxElo_c38(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca aggagaaaaaaccccggatcggcgcgccaccatgtcgttcctcattc gcactcc	88
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactcct tccttttcggttagagcggatttaattaattaaatcgtcttcgtcttgggct	89
pdxElo_c4958(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca aggagaaaaaaccccggatcggcgcgccaccatggcagtggcctt gctcgaggt	90
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactcct tccttttcggttagagcggatttaattaatcaacccctgctgctcccgccta	91
pdxElo_c21679(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca aggagaaaaaaccccggatcggcgcgccaccatgctttcagtttattt ccccgc	92
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactcct tccttttcggttagagcggatttaattaacacgtgcaagcttacccatacg g	93
pdxElo_Irc26016(N	Forward:	94

o)	ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca aggagaaaaaaccccggatcggcgcgccaccatgcccaagcttcc agagatctc Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactcct tccttttcggttagagcggatttaattaattacatcgccttgattttcttgg	95
pKCR_c20574(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca aggagaaaaaaccccggatcggcgcgccaccatgggtctcgacgt gaaggagaa Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactcct tccttttcggttagagcggatttaattaactacgcagcggccttgatctcct	96 97
pKCR_c20772(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca aggagaaaaaaccccggatcggcgcgccaccatggcatctaaagg tggcaattt Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactcct tccttttcggttagagcggatttaattaatcaagcgctcttctcattcttct	98 99
pKCR_c2845(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca aggagaaaaaaccccggatcggcgcgccaccatggcgttggacgt gaaggagaa Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactcct tccttttcggttagagcggatttaattaactactttactccccctttccctt	100 101
pDH_c7190(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca aggagaaaaaaccccggatcggcgcgccaccatgggaggtggca gtaaaagcgg Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactcct tccttttcggttagagcggatttaattaactattcggccttccggctcttcc	102 103
pECR_c41(No)	Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca aggagaaaaaaccccggatcggcgcgccaccatgggcaagcctc agcgagccaa Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactcct tccttttcggttagagcggatttaattaactaaaacccagcgtatcccttg	104 105

	a Forward: ataaaagtatcaacaaaaaattgttaatatacctctatactttaacgtca aggagaaaaaaccccggatcggcgcgccaccatggctagttcagtt	
pd4Des(Mb)	gagaggga	130
	Reverse: aactataaaaaaataaatagggacctagacttcaggttgtctaactcct tccttttcggttagagcggatttaattaattaagcagctctaggcttaactt	131

A list of identified full-length coding sequences is shown in Table 3.

Table 3: Coding polynucleotide sequences, amino acid sequences encoded thereby and expressed sequences (mRNA) of desaturases, elongases or elongase component from Nannochloropsis oculata of the invention.

Gene name	Activity	ORF in bp	SEQ-ID No.	Amino acids	SEQ-ID No.	mRNA in bp	SEQ-ID No.
pd5Des_c738(No)	d5-desaturase	1581	1	526	2	1972	3
pd6Des_c2410(No)	d6-desaturase	1425	4	474	5	1565	6
pd4Des_c5834(No)	d4-desaturase	1527	7	508	8	1963	9
pd8Des_c20493(No)	d8-desaturase	1449	10	482	11	1954	12
pd9Des_c3000(No)	d9-desaturase	1080	13	359	14	1534	15
pd12Des_c6209(No)	d12-desaturase	1317	16	438	17	2049	18
pd15Des_c3421(No)	d15-desaturase	1242	19	413	20	2079	21
pdxElo_c1013(No)	KCS	906	22	301	23	1086	24
pdxElo_c10303(No)	KCS	1023	25	340	26	1894	27
pdxElo_c2186(No)	KCS	1095	28	364	29	1685	30
pdxElo_c2529(No)	KCS	951	31	316	32	1060	33
pdxElo_c37(No)	KCS	831	34	276	35	1302	36

pdxElo_c38(No)	KCS	897	37	298	38	2441	39
pdxElo_c4958(No)	KCS	903	40	300	41	1053	42
pdxElo_c21679(No)	KCS	1485	43	495	44	1755	45
pdxElo_Irc26016(No)	KCS	966	46	321	47	1689	48
pKCR_c20574(No)	KCR	1071	49	356	50	1304	51
pKCR_c20772(No)	KCR	978	52	325	53	1115	54
pKCR_c2845(No)	KCR	1044	55	347	56	1751	57
pDH_c7190(No)	DH	768	58	202	59	1293	60
pECR_c41(No)	ECR	1620	61	539	62	2229	63
pd4Des(Mb)	d4-desaturase	1320	128	439	129	1515	130

Example 4: Activity Assay in Yeast

As an example the activity of identified polypeptides was confirmed by heterologous expression in yeast. Table 4 shows the activity assay of the control yeasts transformed with the empty pYes vector, pYes-pd4Des(Mb), pYes-pd5Des_c738(No) and pYes-pd6Des_c2410(No) construct. In the gas chromatograms of yeast extracts, transformed with pYes-pd4Des(Mb) and fed with 22:4n-6 or 22:5n-3, the d4-desaturated fatty acids 22:5n-6 and 22:6n-3 were detected (Figure 1, Table 4). This result shows that pYes-pd4Des(Mb) has d4-desaturase activtiy. In the gas chromatograms of yeast extracts, transformed with pYes-pd5 Des_c738(No) and fed with 20:3n-6 or 20:4n-3, the d5-desaturated fatty acids 20:4n-6 and 20:5n-3 were detected (Figure 1, Table 4). The fatty acids 20:4n-6 and 20:5n-3 were not present in yeast transformed with the control vector and fed with 20:3n-6 and 20:4n-3. This analysis shows that pYes-pd5 Des_c738(No) has d5-desaturase activity.

Only in the gas chromatograms of yeast extracts, transformed with pYes-pd6Des_c2410(No) and fed with 18:2n-6 or 18:3n-3, the d6-desaturated fatty acids 18:3n-6 and 18:4n-3 were detected (Figure 1, Table 4). This result unambiguously demonstrates that pd6Des_c2410 (No) has d6-desaturase activity. Additionally, the detected 18:4n-3 product suggests that pYes-pd6Des_c2410 (No) has also d15-desaturase activity.

Table 4: Yeast feeding experiment. The substrate and product fatty acid are given as percentage of the total fatty acid pool. The chromatograms of the measurement are shown in figure 1.

Vector	Substrate		Product		Conversion (%)	Activity	Figure
pYes	22:4n-6	73,28	22:5n-6	0,00	0,00	-	1A
pYes	22:5n-3	72,01	22:6n-3	0,00	0,00	-	1B
pYes-pd4Des(Mb)	22:4n-6	66,77	22:5n-6	7,35	9,91	d4Des	1C
pYes-pd4Des(Mb)	22:5n-3	64,10	22:6n-3	7,74	10,78	d4Des	1D
pYes	20:3n-6	89,93	20:4n-6	0,00	0,00	-	1E
pYes	20:4n-3	60,64	20:5n-3	0,00	0,00	-	1F
pd5Des_c738(No)	20:3n-6	85,00	20:4n-6	4,12	4,62	d5Des	1G
pd5Des_c738(No)	20:4n-3	58,89	20:5n-3	6,75	10,29	d5Des	1H
pYes	18:2n-6	20,9	18:3n-6	0,0	0,00	-	1I
pYes	18:3n-3	13,2	18:4n-3	0,0	0,00	-	1J
pYes-pd6Des_c2410(No)	18:2n-6	20,2	18:3n-6	10,6	34,46	d6Des	1K
pYes-pd6Des_c2410(No)	18:2n-6	20,2	18:4n-3	2,0	9,00	d15Des	1K
pYes-pd6Des_c2410(No)	18:3n-3	5,3	18:4n-3	6,9	56,44	d6Des	1L

Additionally the activity of the identified Elo component polypeptides were analyzed. The fatty acids 18:3n-6 and 18:4n-3 were fed to yeasts expressing pdxElo_c37(No) and pdxElo_c1013(No). As a control, yeasts transformed with the empty pYes vector were included in the experiment. In contrast to control-yeasts, yeasts transformed with pYes-pdxElo_c37(No) or pYes-pdxElo_c1013(No) produced 20:3n-6 or 20:4-3, this demonstrates that pdxElo_c37(No) and pdxElo_c1013(No) have d6-Elongase activity.

Table 5: Yeast feeding experiment. The substrate and product fatty acid are given as percentage of the total fatty acid pool.

Vector	Substrate		Product		Conversion (%)	Activity
pYes	18:3n-6	63,87	20:3n-6	0,00	0,00	-
pYes	18:4n-3	71,28	20:4n-3	0,00	0,00	-
pYes-pdxElo_c37(No)	18:3n-6	72,97	20:3n-6	3,35	4,39	d6Elo
pYes-pdxElo_c37(No)	18:4n-3	69,09	20:4n-3	0,77	1,11	d6Elo
pYes-pdxElo_c1013(No)	18:3n-6	70,39	20:3n-6	1,49	2,07	d6Elo

Vector	Substrate		Product		Conversion (%)	Activity
pYes	18:2n-6	42,74	20:2n-6	0,00	0,00	-
pd9Elo_c21679(No	18:2n-6	47,81	20:2n-6	0,54	1,12	d9Elo

Example 5: Expression of Desaturase, KCS, KCR, DH and ECR in Plants.

The novel desaturases, KCS, KCR, DH and ECR from Nannochloropsis oculata can be cloned into a plant transformation vector as described in WO2003/093482, WO2005/083093 or WO2007/093776.

Exemplary suitable combinations of genes for the production of ARA, EPA and DHA are described in table 6, 7, 8 and 9.

Table 6: Gene combinations for the production of arachidonic acid. At least one enzyme with a d12-desaturase, d6-desaturase, d6-elongase and d5-desaturase activity are required for arachidonic acid. Various biosynthetic steps can be catalyzed by enzymes of Nannochloropsis oculata of the present invention.

Activity	Gene	Source organism	SEQ ID NO:
d12-desaturase	d12Des(Ps)	Phytophthora soja	106
	pd12Des_c6209(No)	Nannochloropsis oculata	16
d6-desaturase	d6Des(Ot)	Ostreococcus tauri	108
	pd6Des(No)	Nannochloropsis oculata	4
d6-elongase	d6Elo(Tp)	Thalassiosira pseudonana	110
	d6Elo(Pp)	Physcomitrella patens	112
	pdxElo_c1013(No)	Nannochloropsis oculata	22

	pdxElo_c10303(No)	Nannochloropsis oculata	25
	pdxElo_c2186(No)	Nannochloropsis oculata	28
	pdxElo_c2529(No)	Nannochloropsis oculata	31
	pdxElo_c37(No)	Nannochloropsis oculata	34
	pdxElo_c38(No)	Nannochloropsis oculata	37
	pdxElo_c4958(No)	Nannochloropsis oculata	40
	pdxElo_c21679(No)	Nannochloropsis oculata	43
	pdxElo_lrc26016(No)	Nannochloropsis oculata	46
d5-desaturase	d5Des(Tc)	Thraustochytrium sp.	114
	pd5Des_c738(No)	Nannochloropsis oculata	1
KCR	pKCR_c20574(No)	Nannochloropsis oculata	49
	pKCR_c20772(No)	Nannochloropsis oculata	52
	pKCR_c2845(No)	Nannochloropsis oculata	55
DH	pDH_c7190(No)	Nannochloropsis oculata	58
ECR	pECR_c41(No)	Nannochloropsis oculata	61

Arachidonic acid may be produced by an alterative pathway involving d9-elongase and d8-desaturase activity. Table 7 shows a combination of genes for this pathway.

Table 7: Gene combinations of the alternative pathway for the production of arachidonic acid. Several biosynthetic steps can be catalyzed by enzymes of Nannochloropsis oculata of the present invention.

Activity	Gene	Source organism	SEQ ID NO:
d12-desaturase	d12Des(Ps)	Phytophthora soja	106
	pd12Des_c6209(No)	Nannochloropsis oculata	16
d9-elongase	d9Elo(Ig)	Isochrysis galbana	116
	pdxElo _c21679(No)	Nannochloropsis oculata	43
d8-desaturase	d8Des(Pm)	Perkinsus marinus	113
	pd8Des_c20493(No)	Nannochloropsis oculata	10
d6-elongase	pdxElo_c1013(No)	Nannochloropsis oculata	22
	pdxElo_c10303(No)	Nannochloropsis oculata	25
	pdxElo_c2186(No)	Nannochloropsis oculata	28
	pdxElo_c2529(No)	Nannochloropsis oculata	31
	pdxElo_c37(No)	Nannochloropsis oculata	34
	pdxElo_c38(No)	Nannochloropsis oculata	37
	pdxElo_c4958(No)	Nannochloropsis oculata	40
	pdxElo_c21679(No)	Nannochloropsis oculata	43
	pdxElo_lrc26016(No)	Nannochloropsis oculata	46

d5-desaturase	d5Des(Tc)	Thraustochytrium sp.	114
	pd5Des_c738(No)	Nannochloropsis oculata	1
KCR	pKCR_c20574(No)	Nannochloropsis oculata	49
	pKCR_c20772(No)	Nannochloropsis oculata	52
	pKCR_c2845(No)	Nannochloropsis oculata	55
DH	pDH_c7190(No)	Nannochloropsis oculata	58
ECR	pECR_c41(No)	Nannochloropsis oculata	61

For the production of EPA, the genes listed in table 8 are combined with the genes listed in table 6 or 7.

Table 8: For the production of EPA, in addition to combinations of genes listed in table 6 or 7, the expression of genes of this table are required.

Activity	Gene	Source organism	SEQ ID NO:
d15-desaturase	d15Des(Hr)	Helobdella robusta	120
	pd15Des_c3421(No)	Nannochloropsis oculata	19
omega-3 desaturase	o3Des(Pi)	Phytophthora infestans	122

In addition to the genes of table 5, 6, 7, the genes listed in table 8 are required for the biosynthesis of DHA. These genes allow to elongate EPA by 2 carbon atom and dehydrogenation at the 4th and 5th carbon atom, resulting in the generation of DHA.

Table 9: For the production of DHA, in addition to the genes of table 6 or 7 and 8, the genes of this table are required.

Activity	Gene	Source organism	SEQ ID NO:
d5-elongase	d5Elo(Ot)	Ostreococcus tauri	124
d4-desaturase	d4Des(Tc)	Thraustochytrium sp.	126
	pd4Des_c5834(No)	Nannochloropsis oculata	7
	pd4Des(Mb)	Monosiga brevicollis	128

Transgenic rapeseed lines are 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).

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CLAIMS

1. A polynucleotide comprising a nucleic acid sequence elected from the group consisting of:
   a) a nucleic acid sequence having a nucleotide sequence as shown in SEQ ID NOs: 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, 52, 55, 58, 61 or 128;
   b) a nucleic acid sequence encoding a polypeptide having an amino acid sequence as shown in SEQ ID NOs: 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62 or 129;
   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, KCS, KCR, DH and ECR activity;
   d) a nucleic acid sequence encoding a polypeptide having desaturase, KCS, KCR, DH and ECR activity and having an amino acid sequence which is at least 70% 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, KCS, KCR, DH and ECR activity.

2. The polynucleotide of claim 1, wherein said polynucleotide further comprises an expression control sequence operatively linked to the said nucleic acid sequence.

3. The polynucleotide of claim 1 or 2, wherein said polynucleotide further comprises a terminator sequence operatively linked to the nucleic acid sequence.

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.

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).

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

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:
a) cultivating the host cell of claim 5 under conditions which allow for the production of polyunsaturated fatty acids in said host cell; and
b) obtaining said polyunsaturated fatty acids from the said host cell.

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

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

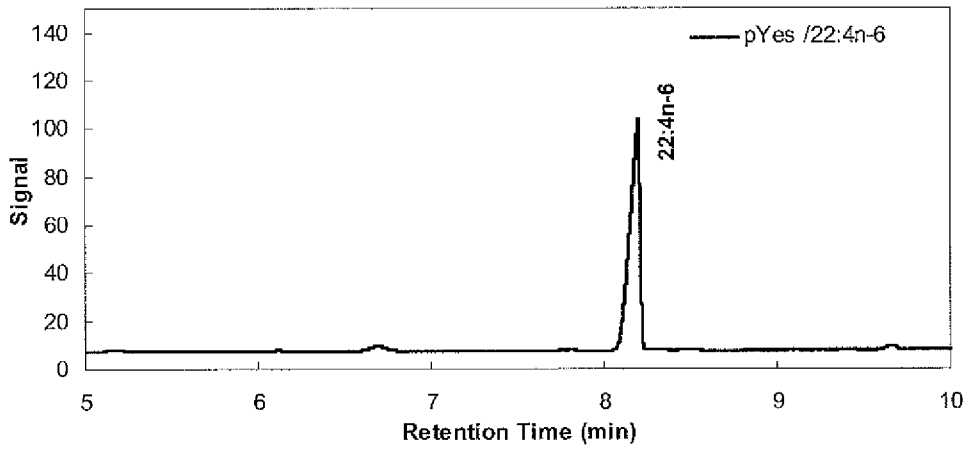
13. A method for the manufacture of an oil, lipid or fatty acid composition comprising the steps of the method of any one of claims 10 to 12 and the further step of formulating the polyunsaturated fatty acid as oil, lipid or fatty acid composition.

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

15. An oil comprising a polyunsaturated fatty acid obtainable by the method of any one of claims 10 to 12.

Fig.

A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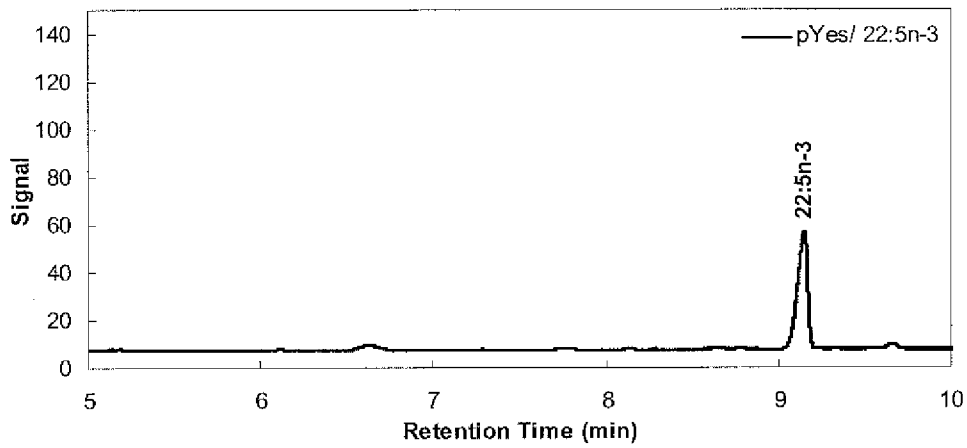
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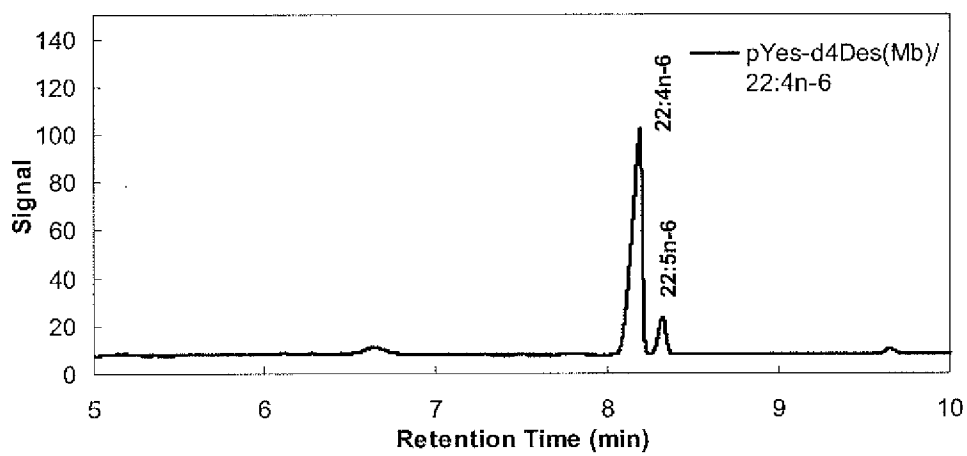
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Fig. cont.

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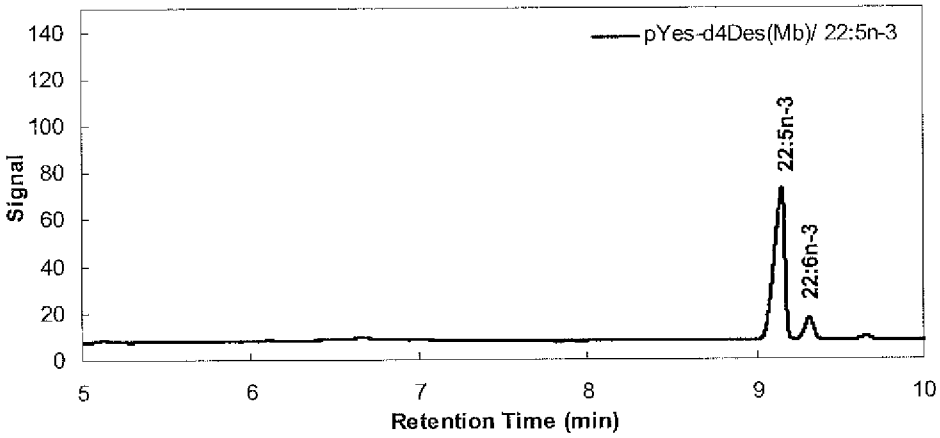
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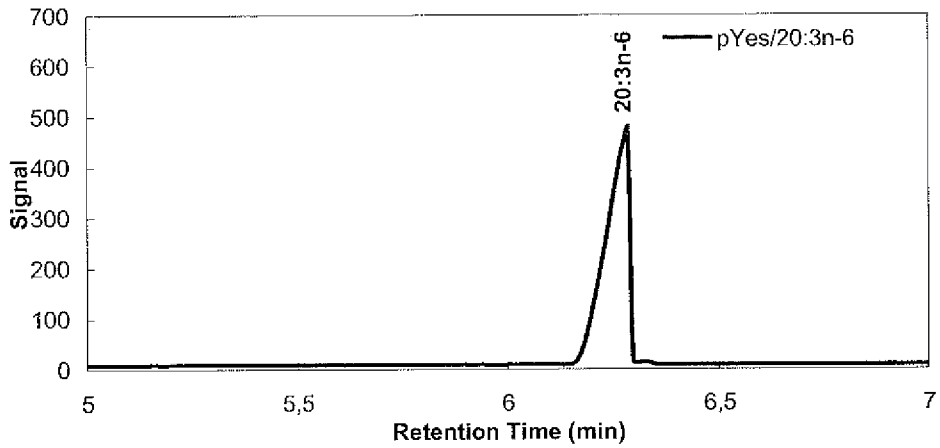
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F

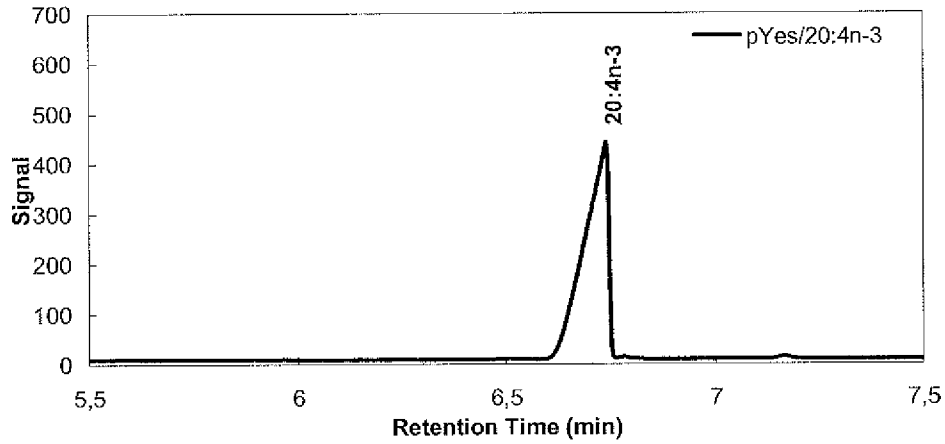
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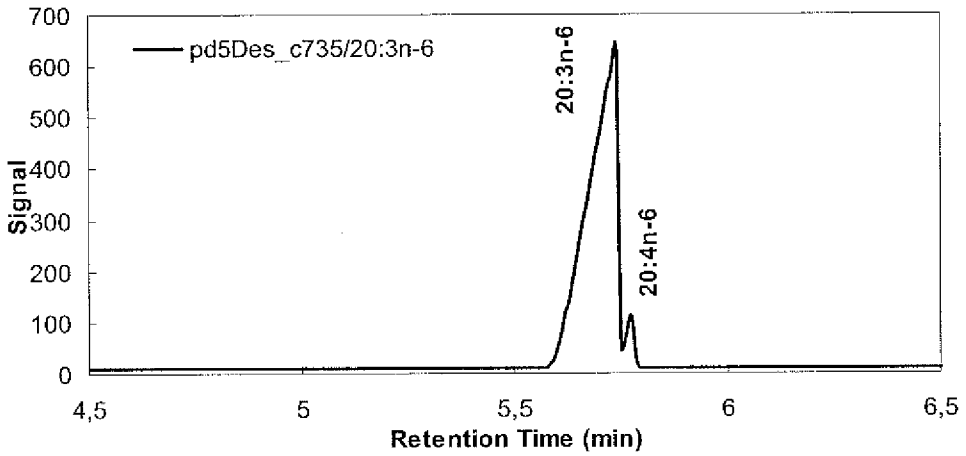
Fig. cont.

G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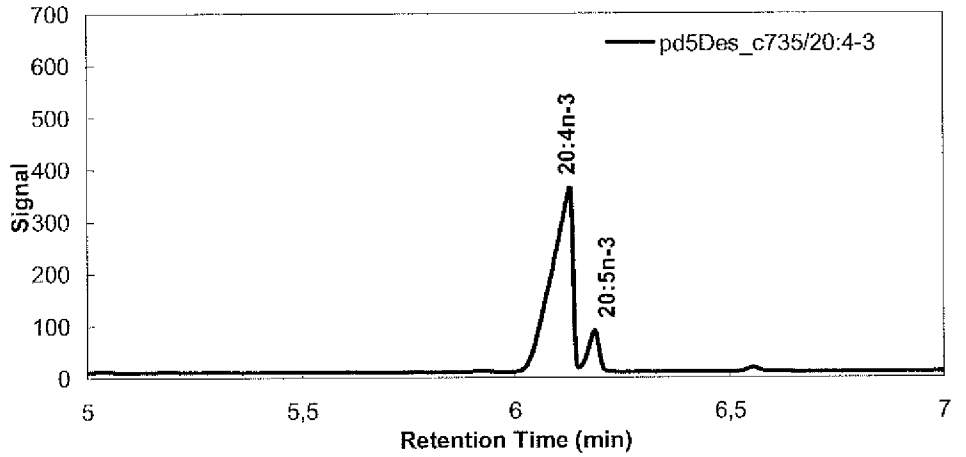
H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700
600
500
400
300
200
100
0
5
5,5
6
6,5
7
Retention Time (min)

I

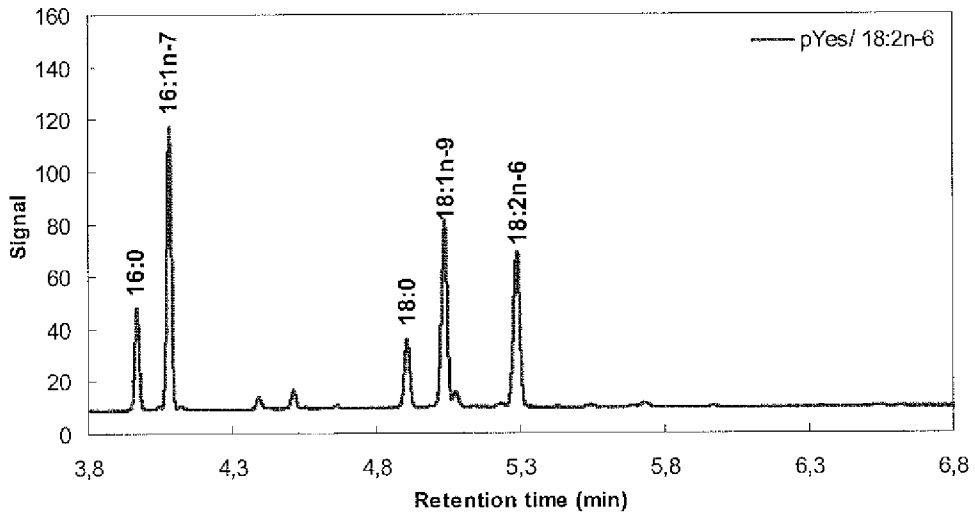
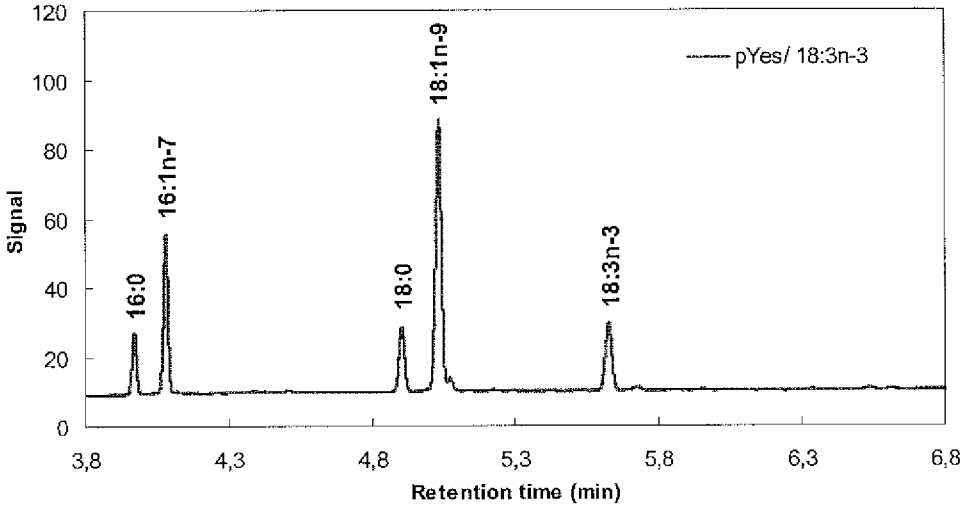

pYes/ 18:2n-6
16:0
16:1n-7
18:0
18:1n-9
18:2n-6
Signal
160
140
120
100
80
60
40
20
0
3,8
4,3
4,8
5,3
5,8
6,3
6,8
Retention time (min)

Fig. cont.

J


120
100
80
60
40
20
0
Signal
16:0
16:1n-7
18:0
18:1n-9
18:3n-3
pYes/ 18:3n-3
3,8
4,3
4,8
5,3
5,8
6,3
6,8
Retention time (min)

K

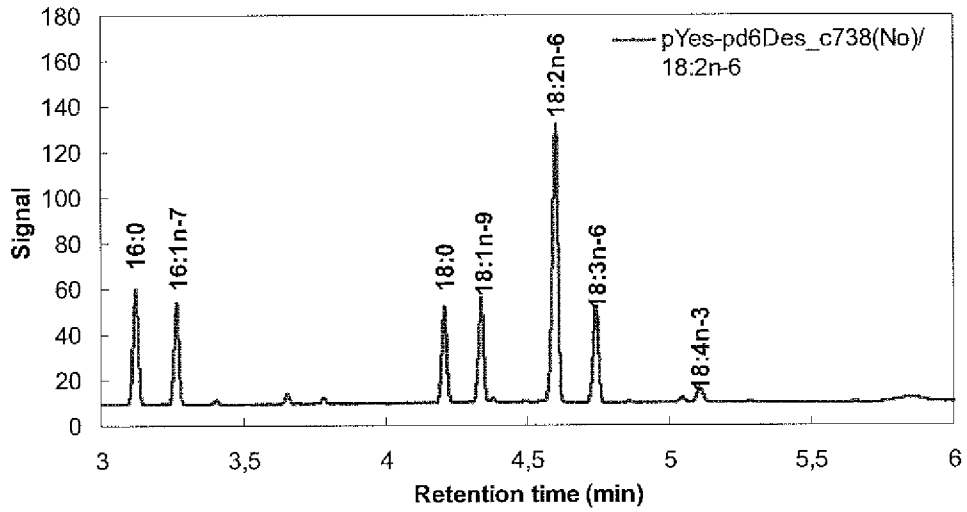
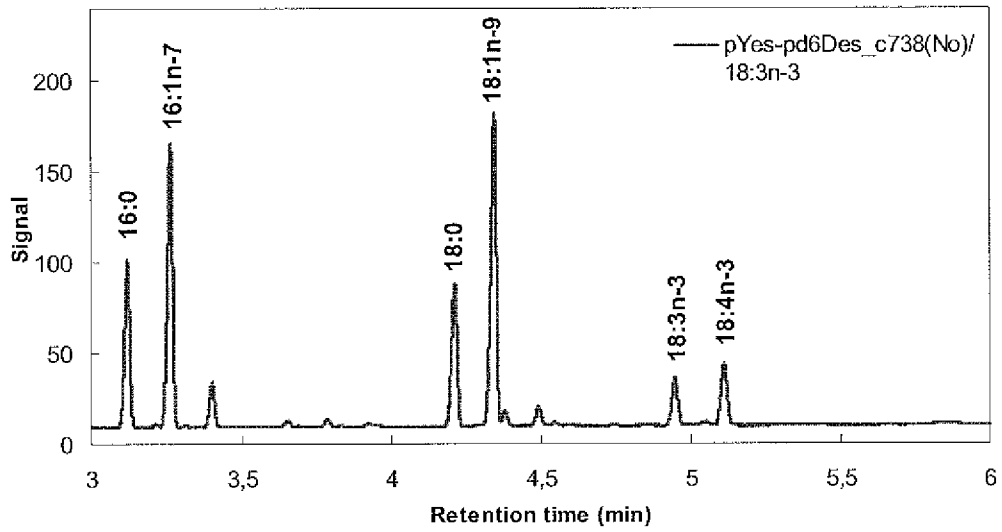

180
160
140
120
100
80
60
40
20
0
Signal
16:0
16:1n-7
18:0
18:1n-9
18:2n-6
18:3n-6
18:4n-3
pYes-pd6Des_c738(No)/
18:2n-6
3
3,5
4
4,5
5
5,5
6
Retention time (min)

Fig. cont.

L


pYes-pd6Des_c738(No)/
18:3n-3
16:0
16:1n-7
18:0
18:1n-9
18:3n-3
18:4n-3
Signal
0
50
100
150
200
3
3,5
4
4,5
5
5,5
6
Retention time (min)