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(54) **Title:** NUCLEIC ACIDS ENCODING DESATURASES AND MODIFIED PLANT OIL

(57) **Abstract:** The present invention relates to nucleic acids derived from Drechslera tritici-repentis, Cylindorcarpon herteronema, Diploida natalensis, Stagonospora nodorum, Microdochium nivalae and Periplaneta americana. The invention also relates to the individual coding sequences and to proteins encoded by these sequences in combination with other sequences as well as to a process for converting oleic acid to linoleic acid to Iinoleic acid and the production of arachidonic acid, eicosapentaenoic acid and/or docosahexaenoic acid in a plant.

Nucleic acids encoding desaturases and modified plant oil

The present invention relates to nucleic acids derived from *Drechslera tritici-repentis, Cylindorcarpon herteronema, Diploids natalensis, Microdochium nivalae, Periplaneta americana* and *Stagonospora nodorum.* The invention also relates to the individual coding sequences and to proteins encoded by these sequences in combination with other sequences as well as to a process for converting oleic acid to linoleic acid to linoleic acid and the production of arachidonic acid, eicosapentaenoic acid and/or docosahexaenoic acid.

Fatty acids and triacylglycerides have a multiplicity of applications in the food industry, in animal nutrition, in cosmetics and in the pharmacological sector. Depending on whether they are free saturated or unsaturated fatty acids or else triacylglycerides with an elevated content of saturated or unsaturated fatty acids, they are suitable for very different applications. Polyunsaturated fatty acids such as linoleic acid and linolenic acid are essential for mammals, since they cannot be produced by the latter. Polyunsaturated ω3-fatty acids and ω6-fatty acids are therefore an important constituent in animal and human nutrition.

Hereinbelow, polyunsaturated fatty acids are referred to as PUFA, PUFAs, LCPUFA or LCPUFAs (**p**oly unsaturated fatty acids, **PUFA,**]png chain **p**oly unsaturated fatty acids, **LCPUFA**).

The various fatty acids and triglycerides are mainly obtained from microorganisms such as Mortierella and Schizochytrium or from oil-producing plants such as soybean, oilseed rape, algae such as Crypthecodinium or Phaeodactylum and others, where they are obtained, as a rule, in the form of their triacylglycerides (= triglycerides = triglycerols). However, they can also be obtained from animals, such as, for example, fish. The free fatty acids are advantageously prepared by hydrolysis. Very long-chain polyunsaturated fatty acids such as docosahexaenoic acid (= DHA, $C22:6^{\Delta 4,7,10,13,16,19}$), eicosapentaenoic acid (= EPA, $C20:5^{\Delta 5,8,11,14,17}$), arachidonic acid (= ARA, $C20:4^{\Delta 5,8,11,14}$), dihomo-γ-linolenic acid ($C20:3^{\Delta 8,11,14}$) or docosapentaenoic acid (DPA, $C22:5^{\Delta 7,10,13,16,19}$) are not synthesized in oil crops such as oilseed rape, soybean, sunflower or safflower. Conventional natural sources of these fatty acids are fish such as herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna, or algae.

Depending on the intended use, oils with saturated or unsaturated fatty acids are preferred. In human nutrition, for example, lipids with unsaturated fatty acids, specifically polyunsaturated fatty acids, are preferred. The polyunsaturated ω3-fatty acids are said to have a positive effect on the cholesterol level in the blood and thus on the possibility of preventing heart disease. The risk of heart disease, stroke or hypertension can be reduced markedly by adding these ω3-fatty acids to the food. Also, ω3-fatty acids have a positive effect on inflammatory, specifically on chronically inflammatory, processes in association with immunological diseases such as rheumatoid arthritis. They are therefore added to foodstuffs, specifically to dietetic foodstuffs, or are employed in medicaments. ω6-Fatty acids such as arachidonic acid tend to have a negative effect on these disorders in connection with these rheumatic diseases on account of our usual dietary intake.

ω3- and ω6-fatty acids are precursors of tissue hormones, known as eicosanoids, such as the prostaglandins, which are derived from dihomo-γ-linolenic acid, arachidonic acid and eicosapentaenoic acid, and of the thromboxanes and leukotrienes, which are derived from arachidonic acid and eicosapentaenoic acid. Eicosanoids (known as the PG2 series) which are formed from ω6-fatty acids generally promote inflammatory reactions, while eicosanoids (known as the PG_3 series) from ω3-fatty acids have little or no proinflammatory effect.

Owing to the positive characteristics of the polyunsaturated fatty acids, there has been no lack of attempts in the past to make available genes which are involved in the synthesis of these fatty acids or triglycerides for the production of oils in various organisms with a modified content of unsaturated fatty acids. Thus, WO 91/13972 and its US equivalent describes a Δ9-desaturase. WO 93/1 1245 claims a Δ15-desaturase and WO 94/1 15 16 a Δ12-desaturase. Further desaturases are described, for example, in EP-A-O 550 162, WO 94/18337, WO 97/30582, WO 97/21340, WO 95/18222, EP-A-O 794 250, Stukey *et* a/., J. Biol. Chem., 265, 1990: 20144-20149, Wada *et* a/., Nature 347, 1990: 200-203 or Huang *et* a/., Lipids 34, 1999: 649-659. However, the biochemical characterization of the various desaturases has been insufficient to date since the enzymes, being membrane-bound proteins, present great difficulty in their isolation and characterization (McKeon *et* a/., Methods in Enzymol. 71, 1981 : 12141-12147, Wang *et ah,* Plant Physiol. Biochem., 26, 1988: 777-792). As a rule, membrane-bound desaturases are characterized by being introduced into a suitable organism which is subsequently analyzed for enzyme activity by analyzing the starting materials and the products. Δ6-Desatu rases are described in WO 93/06712, US 5,614,393, US5614393,

WO 96/21 022, WO 00/21 557 and WO 99/271 11 and the application for the production of fatty acids in transgenic organisms is described in WO 98/46763, WO 98/46764 and WO 98/46765. In this context, the expression of various desaturases and the formation of polyunsaturated fatty acids is also described and claimed in WO 99/6461 6 or WO 98/46776. As regards the expression efficacy of desaturases and its effect on the formation of polyunsaturated fatty acids, it must be noted that the expression of a single desaturase as described to date has only resulted in low contents of unsaturated fatty acids/lipids such as, for example, γ-linolenic acid and stearidonic acid. Moreover, a mixture of ω3- and ω6-fatty acids was obtained, as a rule.

Especially suitable microorganisms for the production of PUFAs are microalgae such as Phaeodactylum tricornutum, Porphiridium species, Thraustochytrium species, Schizochytrium species or Crypthecodinium species, ciliates such as Stylonychia or Colpidium, fungae such as Mortierella, Entomophthora or Mucor and/or mosses such as Physcomitrella, Ceratodon and Marchantia (R. Vazhappilly & F. Chen (1998) Botanica Marina 41: 553-558; K. Totani & K. Oba (1987) Lipids 22: 1060-1062; M. Akimoto *et al.* (1998) Appl. Biochemistry and Biotechnology 73: 269-278). Strain selection has resulted in the development of a number of mutant strains of the microorganisms in question which produce a series of desirable compounds including PUFAs. However, the mutation and selection of strains with an improved production of a particular molecule such as the polyunsaturated fatty acids is a time-consuming and difficult process. This is why recombinant methods as described above are preferred whenever possible.

A variety of synthetic pathways is being discussed for the synthesis of arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (figure 1). Thus, EPA or DHA are produced in marine bacteria such as Vibrio sp. or Shewanella sp. *via* the polyketide pathway (Yu, R. *et al.* Lipids 35:1061-1064, 2000; Takeyama, H. *et al.* Microbiology 143:2725-2731 , 1997).

An alternative strategy is the alternating activity of desaturases and elongases (Zank, T.K. *et al.* Plant Journal 31:255-268, 2002; Sakuradani, E. *et al.* Gene 238:445-453, 1999). A modification of the above-described pathway by Δ6-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase and Δ4-desaturase is the Sprecher pathway (Sprecher 2000, Biochim. Biophys. Acta 1486:21 9-231) in mammals. Instead of the Δ4-desaturation, a further elongation step is effected here to give C_{24}, followed by a further Δ6-desaturation and finally β-oxidation to give the C_{22} chain length. Thus what is known as Sprecher pathway (see figure 1) is, however, not suitable for the production

in plants and microorganisms since the regulatory mechanisms are not known.

Depending on their desaturation pattern, the polyunsaturated fatty acids can be divided into two large classes, viz. ω6- or ω3-fatty acids, which differ with regard to their metabolic and functional activities (fig. 1).

The starting material for the ω6-metabolic pathway is the fatty acid linoleic acid ($18:2^{\Delta 9,12}$) while the ω3-pathway proceeds *via* linolenic acid ($18:3^{\Delta 9,12,15}$). Linolenic acid is formed by the activity of an ω3-desaturase (Tocher *et al.* 1998, Prog. Lipid Res. 37, 73-1 17 ; Domergue *et al.* 2002, Eur. J. Biochem. 269, 4 105-41 13).

Mammals, and thus also humans, have no corresponding desaturase activity (Δ 12- and ω3-desaturase) and must take up these fatty acids (essential fatty acids) *via* the food. Starting with these precursors, the physiologically important polyunsaturated fatty acids arachidonic acid (= ARA, $20:4^{\Delta 5,8,11,14}$), an ω6-fatty acid and the two ω3-fatty acids eicosapentaenoic acid (= EPA, $20:5^{\Delta 5,8,11,14,17}$) and docosahexaenoic acid (DHA, $22:6^{\Delta 4,7,10,13,17,19}$) are synthesized *via* the sequence of desaturase and elongase reactions. The application of ω3-fatty acids shows the therapeutic activity described above in the treatment of cardiovascular diseases (Shimikawa 2001 , World Rev. Nutr. Diet. 88, 100-108), Entzündungen (Calder 2002, Proc. Nutr. Soc. 6 1, 345-358) and Arthritis (Cleland and James 2000, J. Rheumatol. 27, 2305-2307).

The elongation of fatty acids, by elongases, by 2 or 4 C atoms is of crucial importance for the production of C_{20}- and C_{22}-PUFAs, respectively. This process proceeds *via* 4 steps. The first step is the condensation of malonyl-CoA with the fatty-acid-acyl-CoA by ketoacyl-CoA synthase (KCS, hereinbelow referred to as elongase). This is followed by a reduction step (ketoacyl-CoA reductase, KCR), a dehydratation step (dehydratase) and a final reduction step (enoyl-CoA reductase). It has been postulated that the elongase activity affects the specificity and rate of the entire process (Millar and Kunst, 1997 Plant Journal 12:121-131).

There have been a large number of attempts in the past to obtain elongase genes. Millar and Kunst, 1997 (Plant Journal 12:121-131) and Millar *et al.* 1999, (Plant Cell 11:825-838) describe the characterization of plant elongases for the synthesis of monounsaturated long-chain fatty acids (C22:1) and for the synthesis of very long-chain fatty acids for the formation of waxes in plants (C_{28}-C32). Descriptions regarding the synthesis of arachidonic acid and EPA are found, for example, in WO01 59128, WO0012720, WO02077213 and WO0208401 . The synthesis of polyunsaturated C_{24}-

fatty acids is described, for example, in Tvrdik *et al.* 2000, JCB 149:707-71 7 or WO0244320.

Higher plants comprise polyunsaturated fatty acids such as linoleic acid ($18:2^{\Delta 9,12}$) and
5 linolenic acid ($18:3^{\Delta 9,12,15}$). ARA, EPA and DHA are found not at all in the seed oil of higher plants or only in miniscule amounts (E. Ucciani: Nouveau Dictionnaire des Huiles Vegetales [New Dictionary of Vegetable Oils]. Technique & Documentation - Lavoisier, 1995. ISBN: 2-7430-0009-0). However, the production of LCPUFAs in higher plants, preferably in oil crops such as oilseed rape, linseed, sunflower and soybeans,
10 would be advantageous since large amounts of high-quality LCPUFAs for the food industry, animal nutrition and pharmaceutical purposes might be obtained economically. To this end, it is advantageous to introduce, into oil crops, genes which encode enzymes of the LCPUFA biosynthesis *via* recombinant methods and to express them therein. These genes can advantageously be isolated from microorganisms and lower
15 plants which produce LCPUFAs and incorporate them in the membranes or triacylglycerides. Thus, it has already been possible to isolate Δ6-desaturase genes from the moss Physcomitrella patens and Δ6-elongase genes from *P. patens* and from the nematode *C. elegans.*

20 The first transgenic plants which comprise and express genes encoding LCPUFA biosynthesis enzymes and which, as a consequence, produce LCPUFAs were described for the first time, for example, in WO2003/064638, WO2003/093482 (process for the production of polyunsaturated fatty acids in plants). However, these plants produce LCPUFAs in amounts which require further optimization for processing the oils which
25 are present in the plants. Increases of PUFA levels were demonstrated in WO2004/071467 for soybean and in WO2005/083093 for Brassica juncea.

Although biotechnology offers an attractive route for the production of PUFA, current techniques fail to provide an efficient means for the large scale production. Accordingly,
30 there exists a need for an improved and efficient method of production PUFA, such as ARA, EPA and DHA.

The technical problem underlying the present invention could be seen as the provision
35 of means and methods for complying with the aforementioned needs. The technical problem is solved by the embodiments characterized in the claims and herein below.

Thus, the present invention, in principle, contemplates nucleic acid molecules encoding

novel desaturases from the fungi *Drechslera tritici-repentis, Cylindrocarpon heteronema, Diplodia natalensis, Microdochium nivalae, Periplaneta americana* and *Stagonospora nodorum.* In particular the *Drechslera tritici-repentis* delta 12-desaturase, *Diplodia natalensis* delta 12-desaturase, *Microdochium nivalae* delta 12-desaturase, 5 *Periplaneta americana* delta 12-desaturase and *Stagonospora nodorum* delta 12-desaturase, further the *Cylindrocarpon heteronema* delta 15-desaturase and *Microdochium nivalae* d12-desaturase have been identified. Yet further the use of these nucleic acids in the process of manufacturing high levels of ARA, EPA and DHA is provided.

10 Specifically, the present invention pertains to polynucleotides comprising a nucleic acid selected from the group consisting of:

a) a nucleic acid having a nucleic acid sequence as shown in any one of SEQ ID Nos: 1, 3, 5, 7, 31, 33, 35 or 37;

b) a nucleic acid encoding a polypeptide having an amino acid sequence as shown 15 in any one of SEQ ID Nos: 2, 4, 6, 8, 32, 34, 36, or 38;

c) a nucleic acid which has a nucleic acid sequence being at least 60 % identical to the nucleic acid sequence as shown in any one of SEQ ID Nos: 1, 3, 5, 7, 31, 33, 35 or 37, wherein said nucleic acid encodes a polypeptide having desaturase activity;

20 d) a nucleic acid encoding a polypeptide having an amino acid sequence being at least 74.1% identical to the amino acid sequnce shown in any one of SEQ ID Nos: 2, 4, 6, 8, 32, 34, 36, or 38, wherein said nucleic acid encodes a polypeptide having desaturase activity;

e) a nucleic acid which hybridizes under stringent hybridization conditions to the 25 nucleic acid of any one of a) to d), wherein said nucleic acid encodes a polypeptide having desaturase activity;

f) a nucleic acid encoding a fragment of a polypeptide encoded by the nucleic acid sequence of any one of a) to e) having desaturase activity; and

g) a nucleic acid comprising at least 15 contigous nucleotides of the nucleic acid of 30 any one of a) to f).

The term "polynucleotide" as used herein relates to nucleic acid molecules being either DNA or RNA as well as chemically modified derivatives thereof, e.g., biotinylated or methylated nucleic acid molecules. DNA as used herein includes cDNA and genomic 35 DNA. The polynucleotides may be in linear or circular form and may be single or double stranded.

In accordance with the preent invention it has been found that the polynucleotides of

the present invention referred to above encode polypeptides having desaturase activity, i.e. being desaturases.

The term "desaturase" refers to an enzymatic activity which allows for introduction of double bonds in carbohydrates, in general. Particularly preferred in accordance with the present invention are desaturases which introduce a double bond at position 12 or 15 into a fatty acid. More preferably, the desaturase activity referred to in accordance with the present invention is, therefore, a delta 12 or delta 15 desaturase activity. Most preferably, the delta 12 or delta 15 desaturase allows for conversion of subtrates into products as shown in the accompanying Figures, below. Specifically, polynucleotides comprising nucleic acids having a nucleic acid sequence as shown in any one of SEQ ID NOs: 2, 6, 8, 32, or 34, or variants thereof as specified above encode, preferably, desaturases which exhibit delta 12 desaturase activity while polynucleotides comprising nucleic acids having a nucleic acid sequence as shown in any one of SEQ ID NOs: 4 or variants thereof as specified above, preferably, encode desaturases which exhibit delta 15 desaturase activity. Polynucleotides encoding the polypeptide according to SEQ ID NOs: 36 or 38 or the aforementioned variants thereof encode desaturase enzymes. A polypeptide encoded by a polynucleotide can be tested for desaturase activity, preferably, as described in the accompanying Examples below.

The polynucleotides of the present invention encompass also variant polynucleotides of those referred to above by specific nucleic acid or amino acid sequenes. Said variant polynucleotides include homolgs, preferabyl, alleic variants, paralogs or orthologs. Such 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 SEQ ID NO: 1, 3, 5, 7, 31, 33, 35 or 37 by at least one nucleotide substitution, addition and/or deletion whereby the variant nucleic acid sequence shall still encode a polypeptide having desaturase activity as specified above. Variants also encompass nucleic acid molecules comprising a nucleic acid sequence which is capable of hybridizing to the aforementioned specific nucleic acid sequences, preferably, under stringent hybridization conditions. These stringent conditions are known to the skilled worker and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred example for stringent hybridization conditions are hybridization conditions in 6 x sodium chloride/sodium citrate (= SSC) at approximately 65°C, preferably, followed by one or more wash steps in 0.2 x SSC, 0.1% SDS at 50 to 65°C. The skilled worker knows that these hybridization conditions differ depending on the type of nucleic acid and, for example when organic solvents are present, with regard to the temperature and concentration of the buffer. For example, un-

der "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 x SSC (pH 7.2). If organic solvent is present in the abovementioned buffer, for example 50% formamide, the temperature under standard conditions is approximately 42°C.
5 The hybridization conditions for DNA:DNA hybrids are, preferably, 0.1 x 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 x SSC and 30°C to 55°C, preferably between 45°C and 55°C. The abovementioned hybridization temperatures are determined for example for a nucleic acid with approximately 100 bp (= base pairs) in length and a G + C content
10 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;
15 Brown (Ed.) 1991, "Essential Molecular Biology. A Practical Approach", IRL Press at Oxford University Press, Oxford. Alternatively, nucleic acid molecule 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
20 invention may be identified by a sequence comparison of the nucleic acid sequence of the nucleic acid molecule or the amino acid sequence of the polypeptide of the present invention with other desaturase sequences, see also Fig. 2, below. 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
25 animals may be used. Further, variants include nucleic acid molecule comprising nucleic acid sequences which are at least 74.1%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to the nucleic acid sequences shown in SEQ ID NO:
30 1, 3, 5, 7, 31, 33, 35 or 37 retaining desaturase activity. Moreover, also encompassed are nucleic acid molecule which comprise nucleic acid sequences encoding amino acid sequences which are are at least 74.1%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%,
35 at least 99% identical to the amino acid sequences shown in SEQ ID NO: 2, 4, 6, 8 or 32, 34, 36 or 38, wherein the polypeptide comprising the amino acid sequence retains desaturase activity. 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 this context, the algorithms of Needleman and Wunsch or Smith and Waterman give particularly reliable results. To carry out the sequence alignments, the program PileUp (J. Mol. Evolution., 25, 351-360, 1987, Higgins et al., CABIOS, 5 1989:
5 151-153) or the programs Gap and BestFit (Needleman and Wunsch (J. Mol. Biol. 48; 443-453 (1970)) and Smith and Waterman (Adv. Appl. Math. 2; 482-489 (1981))), which are part of the GCG software packet [Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 5371 1 (1991)], are to be used. The sequence identity values recited above in percent (%) are to be determined, preferably, using the pro-
10 gram GAP over the entire sequence region with the following settings: Gap Weight: 50, Length Weight: 3, Average Match: 10.000 and Average Mismatch: 0.000, which, unless otherwise specified, shall always be used as standard settings for sequence alignments.

15 A polynucleotide encoding a biologically active fragment of the polypeptide encoding by the aforementioned polynucleotides of the invention, i.e. a fragment exhibiting desaturase activity as specified above, is also encompassed by the present invention. 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,
20 preferably, comprises at least 15, at least 20, 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 5, at least 10, 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.

25

The variant polynucleotides referred to above, preferably, encode polypeptides retaining 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 activity exhibited by the polypeptide shown in SEQ ID NO: 2, 4, 6, 8 or 32, 34, 36 or 38. The activity may be
30 tested as described in the accompanying Examples.

The nucleic acid molecules 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,
35 the nucleic acid molecule of the present invention may comprise 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 nucleo-

tides of the sequence downstream of the 3' terminus of the coding gene region. Furthermore, the nucleic acid molecule 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 and the other partner of the fusion protein is a
5 heterologous polypeptide. Such fusion proteins may comprise as additional part other enzymes of the fatty acid or lipid 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
10 in the art and comprise FLAG-tags, 6-histidine-tags, MYC-tags and the like.

Variant polynucleotides as referred to in accordance with the present invention may be obtained by various natural as well as artificial sources. For example, nucleic acid molecules may be obtained by in vitro and in vivo mutagenesis approaches using the
15 above mentioned mentioned specific nucleic acid molecules as a basis. Moreover, nucleic acid molecules being homologs or orthologs may be obtained from various animal, plant or fungus species. Preferably, they are obtained from plants such as algae, for example Isochrysis, Mantoniella, Ostreococcus or Crypthecodinium, algae/diatoms such as Phaeodactylum or Thraustochytrium, mosses such as Physcomitrella or Cera-
20 todon, or higher plants such as the Primulaceae such as Aleuritia, Calendula stellata, Osteospermum spinescens or Osteospermum hyoseroides, microorganisms such as fungi, such as Aspergillus, Thraustochytrium, Phytophthora, Entomophthora, Mucor, Drechslera, Diplodia, Microdochium, Periplaneta, Stagonospora, Cylindrocarpon, Microdochium or Mortierella, bacteria such as Shewanella, yeasts or animals. Preferred
25 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
30 from the genera and species Oncorhynchus mykiss, Trutta trutta or Salmo trutta fario. Moreover, the nucleic acid molecules may be obtained from the diatoms such as the genera Thallasiosira or Crypthecodinium.

The polynucleotides of the present invention shall be provided, preferably, either as an
35 isolated nucleic acid molecule (i.e. isolated from its natural context such as a gene locus) or in genetically modified form. An isolated nucleic acid molecule 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 nucleic acid molecule, preferably, is double or single stranded DNA including cDNA or RNA. The term encompasses single as well as double stranded nucleic acid molecules. Moreover, comprised are also chemically modified nucleic acid molecules including naturally occurring modified nucleic acid molecules such as glycosylated or methylated nucleic acid molecules or artificial modified ones such as biotinylated nucleic acid molecules.

Specifically, polynucleotides encoding polypeptides with Δ12-desaturase are variants of the polynucleotide encoding such a desaturase shown in SEQ ID NO: 1 wherein the variants encode polypeptides with at least 75,1% identity at the amino acid level with SEQ ID NO: 2.

Moreover, polynucleotides encoding polypeptides with Δ15-desaturase are variants of the polynucleotide encoding such a desaturase shown in SEQ ID NO: 3 wherein the variants encode polypeptides with at least 74,1% identity at the amino acid level with SEQ ID NO: 4.

Moreover, polynucleotides encoding polypeptides with Δ12-desaturase are variants of the polynucleotide encoding such a desaturase shown in SEQ ID NO: 5 wherein the variants encode polypeptides with at least 74,1% identity at the amino acid level with SEQ ID NO: 6.

Moreover, polynucleotides encoding polypeptides with Δ12-desaturase are variants of the polynucleotide encoding such a desaturase shown in SEQ ID NO: 7 wherein the variants encode polypeptides with at least 95 % identity at the amino acid level with SEQ ID NO: 8.

Moreover, polynucleotides encoding polypeptides with Δ12-desaturase are variants of the polynucleotide encoding such a desaturase shown in SEQ ID NO: 31 wherein the variants encode polypeptides with at least 75 % identity at the amino acid level with SEQ ID NO: 32.

Moreover, polynucleotides encoding polypeptides with Δ15-desaturase are variants of the polynucleotide encoding such a desaturase shown in SEQ ID NO: 33 wherein the variants encode polypeptides with at least 75 % identity at the amino acid level with SEQ ID NO: 34.

Moreover, polynucleotides encoding polypeptides with desaturase are variants of the

polynucleotide encoding such a desaturase shown in SEQ ID NO: 35 or 37 wherein the variants encode polypeptides with at least 75 % identity at the amino acid level with SEQ ID NO: 36 or 38, respectively.

5 In the studies underlying the present invention, advantageously, polynucleotides were identified encoding novel desaturases from various fungi which can be applied for the manufacture of PUFAs and LCPUFAs as specified herein. An overview of the desaturases is given in the following table:

Name	Organism	Activity	SEQ ID NO
D12Des(Dt)	*Drechslera tritici-repentis*	D12-Desaturase	1
D15Des(Cyh)	*Cylindrocarpon heteronema*	D15-Desaturase	3
D12Des(Dn)	*Diplodia natalensis*	D12-Desaturase	5
D12Des(Sn)	*Stagonospora nodorum*	D12-Desaturase	7
D12Des(Mn)	*Microdochium nivalae*	D12-Desaturase	31
D15Des(Mn)	*Microdochium nivalae*	D15-Desaturase	33
dXDes(Pa)	*Periplaneta americana*	Desaturase	35
dXDes(Pa)_2	*Periplaneta americana*	Desaturase	37

10

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
15 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. These techniques are well known to the skilled artisan. Suitable variant polynucleotides of the aforementioned
20 kind can be readily designed based on the structure of the polynucleotides of this invention.

The present invention also contemplates polynucleotides encoding acyltransferases.
25 For further increase of special fatty acids like ARA, EPA and/or DHA or for the increase of overall production of novel fatty acids, such acyltransferases are beneficial. These enzymes are involved in shuffling of different fatty acids from their respective pools of production (phospholipids, Acyl-CoA) or by esterifying the novel fatty acids to different

molecules (diacylglycerol, lysophosphatic acid, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysophosphatidylinositol, lysophosphatidylserine, 1-acyl-phosphatidylcholine etc.). How to apply said enzymes is described in the accompanying Examples and in, e.g., WO2004/076617 or
5 WO2004/087902.

Thus, the invention contemplates acyltransferase-encoding polynucleotides comprising a nucleic acid selected from the group consisting of:

a) a nucleic acid having a nucleic acid sequence as shown in any one of SEQ ID
10 Nos: 102, 104, 106, 108, 110, 112, 135, 137 or 139;

b) a nucleic acid encoding a polypeptide having the amino acid sequence of a polypeptide encoded by a nucleic acid sequence as shown in any one of SEQ ID Nos: 102, 104, 106, 108, 110, 112, 135, 137 or 139;

c) a nucleic acid which has a nucleic acid sequence being at least 60 % identical to
15 the nucleic acid sequence specififed in a), wherein said nucleic acid encodes a polypeptide having acyltransferase activity;

d) a nucleic acid encoding a polypeptide having an amino acid sequence being at least 60% identical to the amino acid sequnce encoded by a nucleic acid sequence as shown in any one of SEQ ID Nos: 102, 104, 106, 108, 110, 112, 135,
20 137 or 139, wherein said nucleic acid encodes a polypeptide is having acyltransferase activity;

e) a nucleic acid which hybridizes under stringent hybridization conditions to the nucleic acid of any one of a) to d), wherein said nucleic acid encodes a polypeptide having desaturase activity;

25 f) a nucleic acid encoding a fragment of a polypeptide encoded by the nucleic acid sequence of any one of a) to e) having acyltransferase activity; and

g) a nucleic acid comprising at least 15 contigous nucleotides of the nucleic acid of any one of a) to f).

30 The definitions made above for the desaturase-encoding polynucleotides of the invention apply mutatis mutandis to the acyltransferase-encoding polynucleotides.

The term "acyltransferase activity" or "acyltransferase" as used herein encompasses all enymatic activities and enzymes which are capable of transferring or are involved in
35 the transfer of PUFA and, in particular; LCPUFA from the acly-CoA pool or the membrane phospholipis to the triglycerides, from the acyl-CoA pool to membrane lipids and from membrane lipids to the acyl-CoA pool by a transesterification process. It will be understood that this acyltransferase activity will result in an increase of the LCPUFA

esterified to triglycerides in, e.g., seed oils. In particular, it is envisaged that these acyltransferases are capable of producing triglycerides having esterified ARA, EPA or even DHA, or that these acyltransferases are capable of enhancing synthesis of desired PUFA by increasing the flux for specific intermediates of the desired PUFA between
5 the acyl-CoA pool (the site of elongation) and membrane lipids (the predominant site of desaturation). Specifically, acyltransferase activity as used herein pertains to lysophospholipid acyltransferase (LPLAT) activity, preferably, to Lysophosphophatidylethanolamine acyltransferase (LPEAT) activity. Specifically, the aclytransferase encoding nucleic acid sequences as shown in SEQ ID NOs: 102, 104, and 106 as well as 135, 137
10 and 139 encode LPLAT activity. The aclytransferase encoding nucleic acid sequences as shown in SEQ ID NOs: 108, 110, and 112 encode LPEAT activity.

The acyltransferase polynucleotides of the present invention encompass also variant polynucleotides of those referred to above by specific nucleic acid or amino acid se-
15 quenes. Said variant polynucleotides include homolgs, preferabyl, alleic variants, paralogs or orthologs. Such 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 SEQ ID NO: 102, 104, 106, 108, 110, 112, 135, 137 or 139by at least one nucleotide substitution, addition and/or deletion
20 whereby the variant nucleic acid sequence shall still encode a polypeptide having desaturase activity as specified above. Variants also encompass nucleic acid molecules comprising a nucleic acid sequence which is capable of hybridizing to the aforementioned specific nucleic acid sequences, preferably, under stringent hybridization conditions. Alternatively, nucleic acid molecule variants are obtainable by PCR-based tech-
25 niques as specified elsewhere herein. Further, variants include nucleic acid molecule comprising nucleic acid sequences which are at least 60%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to the nucleic acid sequences shown in SEQ
30 ID NO: 102, 104, 106, 108, 110, 112, 135, 137 or 139 retaining acyltransferase activity. Moreover, also encompassed are nucleic acid molecule which comprise nucleic acid sequences encoding amino acid sequences which are are at least 60%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least
35 96%, at least 97%, at least 98%, at least 99% identical to the amino acid sequences encoded by the nucleic acid sequences shown in SEQ ID NO: 102, 104, 106, 108, 110, 112, 135, 137 or 139, wherein the polypeptide comprising the amino acid sequence retains acyltransferase activity.

A polynucleotide encoding a biologically active fragment of the polypeptide encoding by the aforementioned polynucleotides of the invention, i.e. a fragment exhibiting acyl-transferase activity as specified above, is also encompassed by the present invention.

The variant polynucleotides referred to above, preferably, encode polypeptides retaining 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 acyltransferase activity exhibited by the polypeptide encoded by a nucleic acid sequence as shown in SEQ ID NO: 102, 104, 106, 108, 110, 112, 135, 137 or 139. The activity may be tested as described in the accompanying Examples.

The present invention contemplates a vector comprising the polynucleotide of the present invention (i.e. the desaturase or acyltransferase encoding polynucleotides).

The term "vector", preferably, encompasses phage, plasmid, viral or retroviral 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
5 in vitro using an appropriate packaging cell line prior to application to host cells. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host/cells.

Preferably, the vector referred to herein is suitable as a cloning vector, i.e. replicable in
10 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
15 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
20 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
25 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
30 Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida), chapter 6/7, pp. 71-1 19 (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
35 (1993), 128-143; Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991), 205-225.

More preferably, the vector of the present invention is an expression vector. In such an

expression vector, the nucleic acid molecule is operatively linked to expression control sequences (also called "expression cassette") allowing expression in prokaryotic or eukaryotic cells or isolated fractions thereof. Expression of said polynucleotide comprises transcription of the nucleic acid molecule, preferably, into a translatable mRNA.
5 Regulatory elements ensuring expression in eukaryotic cells, preferably mammalian cells, are well known in the art. They, preferably, comprise regulatory sequences ensuring initiation of transcription and, optionally, poly-A signals ensuring termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers. Possible regulatory elements
10 permitting expression in prokaryotic host cells comprise, e.g., the *lac, trp* or *tac* promoter in *E. coli,* and examples for regulatory elements permitting expression in eukaryotic host cells are the *A0X1* or *GAL1* promoter in yeast or the CMV-, SV40-, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells. Moreover, inducible expression control sequences
15 may be used in an expression vector encompassed by the present invention. Such inducible vectors may comprise *tet* or *lac* operator sequences or sequences inducible by heat shock or other environmental factors. Suitable expression control sequences are well known in the art. Beside elements which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination
20 signals, such as the SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. Preferably, the expression vector is also a gene transfer or targeting vector. Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of the nucleic acid molecule or vector of the invention into targeted cell population. Meth-
25 ods which are well known to those skilled in the art can be used to construct recombinant viral vectors; see, for example, the techniques described in Sambrook, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory (1989) N.Y. and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1994).

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Suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pCDMδ, pRc/CMV, pcDNAI , pcDNA3 (Invitrogene) or pSPORTI (GIBCO BRL). Further examples of typical fusion expression vectors are pGEX (Pharmacia Biotech Inc; Smith, D.B., and Johnson, K.S. (1988) Gene
35 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 et

al. (1988) Gene 69:301-31 5) and pET 11d (Studier et al., Gene Expression Technol¬ogy: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 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
5 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 poly¬merase 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 pro-
10 karyotic organisms; these vectors are, for example, in E. coli, pLG338, pACYC184, the pBR series such as pBR322, the pUC series such as pUC18 or pUC19, the M113mp series, pKC30, pRep4, pHS1 , pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-1111 13-B1, λgt1 1 or pBdCI, in Streptomyces pIJ101 , pIJ364, pIJ702 or pIJ361 , in Bacillus pUB1 10, pC194 or pBD214, in Corynebacterium pSA77 or pAJ667. Examples of vec-
15 tors for expression in the yeast S. cerevisiae comprise pYeDesaturased (Baldari et al. (1987) Embo J. 6:229-234), pMFa (Kurjan and Herskowitz (1982) Cell 30:933-943), pJRY88 (Schultz et al. (1987) Gene 54:1 13-123) and pYES2 (Invitrogen Corporation, San Diego, CA). Vectors and processes for the construction of vectors which are suit¬able for use in other fungi, such as the filamentous fungi, comprise those which are
20 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 ,
25 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 et al. (1983) Mol. Cell Biol. 3:21 56-21 65) and the pVL series (Lucklow and Summers (1989) Virology 170:31-39).
30

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, Ipp, lac, Ipp-lac, laclq, T7, T5, T3, gal, trc, ara, SP6, λ-PR or λ-PL promoters are, pref¬erably, used in Gram-negative bacteria. For Gram-positive bacteria, promoters amy
35 and SPO2 may be used. From yeast or fungal promoters ADC1 , MFa, AC, P-60, CYC1 , GAPDH, TEF, rp28, ADH are, preferably, used or from plant the promoters CaMV/35S [Franck et al., Cell 21 (1980) 285-294], PRP1 [Ward et al., Plant. Mol. Biol. 22 (1993)], SSU, OCS, lib4, usp, STLS1 , B33, nos or the ubiquitin or phaseolin pro-

moter. Also preferred in this context are inducible promoters, such as the promoters described in EP A 0 388 186 (benzylsulfonamide-inducible), Plant J. 2, 1992:397-404 (Gatz et al., tetracyclin-inducible), EP A 0 335 528 (abscisic-acid-inducible) or WO 93/21334 (ethanol- or cyclohexenol-inducible). Further suitable plant promoters are the
5 promoter of cytosolic FBPase or the ST-LSI promoter from potato (Stockhaus et al., EMBO J. 8, 1989, 2445), the phosphoribosyl-pyrophosphate amidotransferase promoter from Glycine max (Genbank accession No. U87999) or the node-specific promoter described in EP-A-O 249 676. Particularly preferred are promoters which enable the expression in tissues which are involved in the biosynthesis of fatty acids. Also par-
10 ticularly 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 advantageous 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
15 (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 example for monocots: lpt-2 or lpt-1 promoter from barley (WO 95/15389 and WO 95/23230), hordein promoter from barley
20 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 de-
25 scribed in WO 99/16890.

The polynucleotide of the present invention can be expressed in single-cell plant cells (such as algae), see Falciatore et al., 1999, Marine Biotechnology 1 (3):239-251 and the references cited therein, and plant cells from higher plants (for example Spermato-
30 phytes, such as arable crops) by using plant expression vectors. Examples of plant expression vectors comprise those which are described in detail in: Becker, D., Kemper, E., Schell, J., and Masterson, R. (1992) "New plant binary vectors with selectable markers located proximal to the left border", Plant Mol. Biol. 20:1 195-1 197; and Bevan, M.W. (1984) "Binary Agrobacterium vectors for plant transformation", Nucl. Ac-
35 ids Res. 12:871 1-8721 ; Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, p. 15-38. A plant expression cassette, preferably, comprises regulatory sequences which are capable of controlling the gene expression in plant cells and which

are functionally linked so that each sequence can fulfill its function, such as transcriptional termination, for example polyadenylation signals. Preferred polyadenylation signals are those which are derived from Agrobacterium tumefaciens T-DNA, such as the gene 3 of the Ti plasmid pTiACH5, which is known as octopine synthase (Gielen et al.,
5 EMBO J. 3 (1984) 835 et seq.) 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 mo-
10 saic virus leader sequence, which increases the protein/RNA ratio (Gallie et al., 1987, Nucl. Acids Research 15:8693-871 1). As described above, plant gene expression must be functionally linked to a suitable promoter which performs the expression of the gene in a timely, cell-specific or tissue-specific manner. Promoters which can be used are constitutive promoters (Benfey et al., EMBO J. 8 (1989) 2195-2202) such as those
15 which are derived from plant viruses such as 35S CAMV (Franck et al., Cell 21 (1980) 285-294), 19S CaMV (see also US 5352605 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
20 into its relevant cell compartment (for a review, see Kermode, Crit. Rev. Plant Sci. 15, 4 (1996) 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
25 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 et al. (1992) Plant J. 2, 397-404) and
30 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 et al., Plant Mol. Biol. 22 (1993) 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 A 0 375 091). The
35 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 5,608,1 52), the USP

promoter from Vicia faba (Baeumlein et al., Mol. Gen. Genet., 1991 , 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 et al., 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, J., Fritsch, E.F., and Maniatis, T., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

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.

The present invention, furthermore, relates to a host cell comprising the polynucleotide of the invention or which is transformed with the vector of the 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.), sun-

flower *(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
5 perilla.

Also preferably, said host cell is a microbial cell. More preferably, said microbial cell is selected from the group consisting of *Candida, Cryptococcus, Lipomyces, Rhodosporidium, Yarrowia, Thraustochytrium, Pythium, Schizochytrium* and *Crythe-*
10 *codinium.*

The present invention relates to a plant or plant seed comprising the nucleic acid mole¬cule of the invention, the vector of the invnetion or the host cell of the invention.

15 Preferred plants to be used for introducing the polynucleotide or the vector of the inven¬tion 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. Advantageous plants are selected from the group of the plant families
20 Adelotheciaceae, Anacardiaceae, Asteraceae, Apiaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Caricaceae, Cannabaceae, Convolvulaceae, Chenopodiaceae, Crypthecodiniaceae, Cucurbitaceae, Ditrichaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Geraniaceae, Gramineae, Juglandaceae, Lauraceae, Leguminosae, Linaceae, Prasinophyceae or vegetable plants or ornamentals such as
25 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
30 genera Calendula, Carthamus, Centaurea, Cichorium, Cynara, Helianthus, Lactuca, Locusta, Tagetes, Valeriana, for example the genus and species Calendula officinalis [common marigold], Carthamus tinctorius [saff lower], *Centaurea cyanus* [cornflower], *Cichorium intybus* [chicory], Cynara scolymus [artichoke], *Helianthus annus* [sunflower], *Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola L. ssp.*
35 *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 ca-*

rota [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 cylindήcus 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 berteήanum, 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 austale, Physcomitrium californicum, Physcomitrium collenchymatum, Physcomitrium coloradense, Physcomitrium cupuliferum, Physcomitrium drummondii, Physcomitrium eurystomum, Physcomitrium flexifolium, Physcomitrium hookeri, Physcomitrium hookeri var. serratum, Physcomitrium immersum, Physcomitrium keller-*

manii, 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 [illegible] *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 austɧacum, 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, Hor-*

deum secalinum, Hordeum distichon, Hordeum aegiceras, Hordeum hexastichon, Hordeum hexastichum, Hordeum irregulare, Hordeum sativum, Hordeum secalinum [barley], *Secale cereale* [rye], [illegible] *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,
5 Tagetes, Solanaceae plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa or bushy plants (coffee, cacao, tea), SaNx species, and perennial grasses and fodder crops. Preferred plants according to the invention are oil crop plants such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, Calendula, Punica, evening primrose, pumpkin/squash,
10 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.

15

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, Phy-
20 tophthora, Ceratodon, Isochrysis, Aleurita, Muscarioides, Mortierella, Phaeodactylum, Cryphthecodinium, specifically from the genera and species Thallasiosira pseudonona, Euglena gracilis, Physcomitrella patens, Phytophtora infestans, Fusarium graminaeum, Cryptocodinium cohnii, Ceratodon purpureus, Isochrysis galbana, Aleurita farinosa, Thraustochytrium sp., Muscarioides viallii, Mortierella alpina, Phaeodactylum tricornu-
25 turn or Caenorhabditis elegans or especially advantageously Phytophtora infestans, Thallasiosira pseudonona and Cryptocodinium cohnii.

Transgenic plants may be obtained by transformation techniques as published, and cited, in: Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida),
30 chapter 6/7, pp.71-1 19 (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, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991),
35 205-225. 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.

The host cell of the present invention, more preferably, is capable of producing the unsaturated fatty acids specified in detail below. To this end, further enzymes including other desaturases or elongases may be reqired dependent on the enzymatic setup which is endogenously present in the host cell. It is to be understood that further enzymes which might be required for the production of the unsaturated fatty acid may be exogenously supplied, e.g., by transforming the host cell with expressible polynucleotides encoding said enzymes. Alternatively, the activity of undesired endogenously present enzymes may be inhibited, e.g., by applying anti-sense nucleic acids, ribozymes, siRNA molecules, morpholino nucleic acids (phosphorodiamidate morpholino oligos), triple-helix forming oligonucleotides, inhibitory oligonucleotides, or micro RNA molecules. In particular, the host cell envisaged by the present invention, more preferably, comprises at least one enzymatic activity selected from the group consisting of: Δ-4-desaturase, Δ-5-desaturase, Δ-5-elongase, Δ-6-desaturase, Δ12-desaturase, Δ15-desaturase, ω3-desaturase and Δ-6-elongase activity. Moreover, it will be understood that the desaturases and acyltransferases of the present invention may be co-expressed in order to increase the PUFA production and, in particular for ARA, EPA and/or DHA manufacture.

The present invention also provides for a method of producing a polypeptide comprising culturing the host cell of the invention in an appropriate culture medium to, thereby, produce the polypeptide encoded by a polynucleotide of the invention.

The 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. A polypeptide obtained by the said method includes variant polypeptides which are post-translationally modified, e.g., phosphorylated or myristylated, or which are processed on either RNA or protein level.

In principle, the present invention, however, relates to a polypeptide encoded by the polynucleotide of the present invention.

The term "polypeptide" as used herein encompasses essentially purified polypeptides or polypeptide preparations comprising other proteins in addition. Moreover, the term also, preferably, includes polypeptides which are present in a host cell, plant or plant seed wherein the said host cell, plant or plant seed is not the biological source in which
5 the polypeptide occurs naturally. 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. The terms
10 "polypeptide", "peptide" or "protein" are used interchangeable throughout this specification. As referred to above, the polypeptide of the present invention shall exhibit dehydratase activity and, thus, can be used for the manufacture of unsaturated fatty acids, either in a host cell or in a transgenic animal or plant as described elsewhere in this specification.

15

The present invention also relates to an antibody which specifically recognizes the polypeptide of the present invention.

Antibodies against the polypeptides of the invention can be prepared by well known
20 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
25 monoclonal antibody, a polyclonal antibody, a single chain antibody, a human or humanized antibody or primatized, chimerized or fragment thereof. Also comprised as antibodies by the present invention are a bispecific antibody, a synthetic antibody, an antibody fragment, such as Fab, Fv or scFv fragments etc., or a chemically modified derivative of any of these. The antibody of the present invention shall specifically bind
30 (i.e. does 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
35 Spring Harbor, 1988. Monoclonal antibodies can be prepared by the techniques originally described in Kohler and Milstein, Nature 256 (1975), 495, and Galfre, Meth. Enzymol. 73 (1981), 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 compounds interacting with the proteins according to the invention.

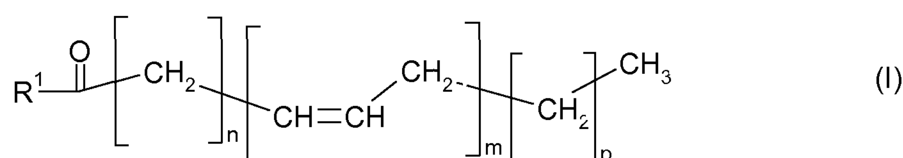
Encompassed by the present invention is a method for manufacturing an unsaturated fatty acid, comprising culturing the host cell of the invention or the plant or plant seed of the invention such that the unsaturated fatty acid is produced. It is to be understood that, preferably, the method may comprise further steps which are required for isolating the unsaturated fatty acid from the host cell.

Also contemplated is a method of modulating the production of an unsaturated fatty acid comprising culturing the host cell of the invention or the plant or plant seed of the invention, such that modulation of the production of an unsaturated fatty acid occurs.

In a preferred embodiment of the methods of the present invention, said methods further comprises the step of recovering the unsaturated fatty acid from said culture.

The present invention, further, relates to a method of manufacturing an unsaturated fatty acid comprising contacting a composition comprising at least one desaturase target molecule with at least one polypeptide of the invention under conditions such that the unsaturated fatty acid is produced.

The term "unsaturated fatty acid" or "elongated fatty acid" as used herein, preferably, encompasses compounds having a structure as shown in the general formula I

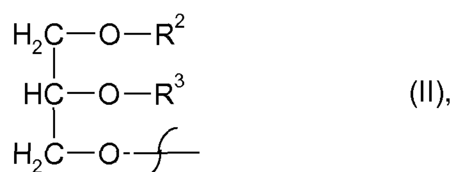


(I)

wherein the variables and substituents in formula I are

R^1 = hydroxyl, coenzyme A (thioester), lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidylglycerol, lyso-

phosphatidylserine, lysophosphatidylinositol, sphingo base or a radical of the formula II



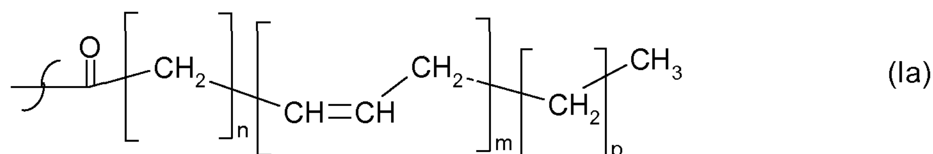
(II),

5

R^2 = hydrogen, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidylglycerol, lysophosphatidylserine, lysophosphatidylinositol or saturated or unsaturated C_2-C24-alkylcarbonyl,

R^3 = hydrogen, saturated or unsaturated C_2-C_{24}-alkylcarbonyl, or R^2 and R^3 independently of each other are a radical of the formula Ia:

10



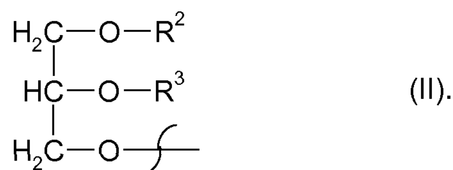
(Ia)

n = 2, 3, 4, 5, 6, 7 or 9, m = 2, 3, 4, 5 or 6 and p = 0 or 3;

15

Preferably, R^1 in the general formula I is hydroxyl, coenzyme A (thioester), ysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidylglycerol, lysophosphatidylserine, lysophosphatidylinositol, sphingo base or a radical of the formula II

20



(II).

The abovementioned radicals of R^1 are always bonded to the compounds of the general formula I in the form of their thioesters.

25

Preferably, R^2 in the general formula II is hydrogen, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidylglycerol, lysophosphatidylserine, lysophosphatidylinositol or saturated or unsaturated C_2-C_{24}-alkylcarbonyl. Moreover, alkyl radicals which may be mentioned are substituted or

unsubstituted, saturated or unsaturated C_2-C_{24}-alkylcarbonyl chains such as ethylcarbonyl, n-propylcarbonyl, n-butylcarbonyl, n-pentylcarbonyl, n-hexylcarbonyl, n-heptylcarbonyl, n-octylcarbonyl, n-nonylcarbonyl, n-decylcarbonyl, n-undecylcarbonyl, n-dodecylcarbonyl, n-tridecylcarbonyl, n-tetradecylcarbonyl, n-pentadecylcarbonyl, n-hexadecylcarbonyl, n-heptadecylcarbonyl, n-octadecylcarbonyl-, n-nonadecylcarbonyl, n-eicosylcarbonyl, n-docosanylcarbonyl- or n-tetracosanylcarbonyl, which comprise one or more double bonds. Saturated or unsaturated Cio-C22-alkylcarbonyl radicals such as n-decylcarbonyl, n-undecylcarbonyl, n-dodecylcarbonyl, n-tridecylcarbonyl, n-tetradecylcarbonyl, n-pentadecylcarbonyl, n-hexadecylcarbonyl, n-heptadecylcarbonyl, n-octadecylcarbonyl, n-nonadecylcarbonyl, n-eicosylcarbonyl, n-docosanylcarbonyl or n-tetracosanylcarbonyl, which comprise one or more double bonds, are preferred. Preferred are saturated and/or unsaturated Ci_0-C_{22}-alkylcarbonyl rad icals such as Cio-alkylcarbonyl, Cn-alkylcarbonyl, Ci_2-alkylcarbonyl, Ci_3-alkylcarbonyl, Ci_4-alkylcarbonyl, Ci_6-alkylcarbonyl, Ciβ-alkylcarbonyl, C_2o-alkylcarbonyl or C_{22}-alkylcarbonyl radicals which comprise one or more double bonds. Particularly preferred are saturated or unsaturated C_2o-C_{22}-alkylcarbonyl radicals such as C_2o-alkylcarbonyl or C_{22}-alkylcarbonyl radicals which comprise one or more double bonds. These preferred radicals can comprise two, three, four, five or six double bonds. The particularly preferred radicals with 20 or 22 carbon atoms in the fatty acid chain comprise up to six double bonds, advantageously two, three, four or five double bonds, especially preferably two, three or four double bonds. All the abovementioned radicals are derived from the corresponding fatty acids.

Preferably, R^3 in the formula II is hydrogen, saturated or unsaturated C_2-C24-alkylcarbonyl. Alkyl radicals which may be mentioned are substituted or unsubstituted, saturated or unsaturated C_2-C_{24}-alkylcarbonyl chains such as ethylcarbonyl, n-propylcarbonyl, n-butylcarbonyl-, n-pentylcarbonyl, n-hexylcarbonyl, n-heptylcarbonyl, n-octylcarbonyl, n-nonylcarbonyl, n-decylcarbonyl, n-undecylcarbonyl, n-dodecylcarbonyl, n-tridecylcarbonyl, n-tetradecylcarbonyl, n-pentadecylcarbonyl, n-hexadecylcarbonyl, n-heptadecylcarbonyl, n-octadecylcarbonyl-, n-nonadecylcarbonyl, n-eicosylcarbonyl, n-docosanylcarbonyl- or n-tetracosanylcarbonyl, which comprise one or more double bonds. Saturated or unsaturated Ci_0-$C_2$2-alkylcarbonyl radicals such as n-decylcarbonyl, n-undecylcarbonyl, n-dodecylcarbonyl, n-tridecylcarbonyl, n-tetradecylcarbonyl, n-pentadecylcarbonyl, n-hexadecylcarbonyl, n-heptadecylcarbonyl, n-octadecylcarbonyl, n-nonadecylcarbonyl, n-eicosylcarbonyl, n-docosanylcarbonyl or n-tetracosanylcarbonyl, which comprise one or more double bonds, are preferred. Preferred are saturated and/or unsaturated Ci_0-C_{22}-alkylcarbonyl rad ical s such as Cio-alkylcarbonyl, Cn-alkylcarbonyl, Ci_2-alkylcarbonyl, C13-

alkylcarbonyl , Ci_4-alkylcarbonyl, Ci_6-alkylcarbonyl, $Ci\beta$-alkylcarbonyl, C_2o-alkylcarbonyl or C_{22}-alkylcarbonyl radicals which comprise one or more double bonds. Particularly preferred are saturated or unsaturated C_2o-C_{22}-alkylcarbonyl radicals such as C_2o-alkylcarbonyl or C_{22}-alkylcarbonyl radicals which comprise one or more double bonds. These preferred radicals can comprise two, three, four, five or six double bonds. The particularly preferred radicals with 20 or 22 carbon atoms in the fatty acid chain comprise up to six double bonds, advantageously two, three, four or five double bonds, especially preferably two, three or four double bonds. All the abovementioned radicals are derived from the corresponding fatty acids.

The abovementioned radicals of R^1, R^2 and R^3 can be substituted by hydroxyl and/or epoxy groups and/or can comprise triple bonds.

The unsaturated fatty acids according to the present invention are, preferably, polyunsaturated fatty acids (PUFAs). The polyunsaturated fatty acids according to the invention advantageously comprise at least two, advantageously three, four, five or six, double bonds. The fatty acids especially advantageously comprise two, three, four or five double bonds. Unsaturated fatty acids, preferably, comprise 20 or 22 carbon atoms in the fatty acid chain. Saturated fatty acids are advantageously reacted to a minor degree, or not at all, by the nucleic acids used in the process. To a minor degree is to be understood as meaning that the saturated fatty acids are reacted with less than 5% of the activity, advantageously less than 3%, especially advantageously with less than 2% of the activity in comparison with polyunsaturated fatty acids. These fatty acids which have been produced can be produced in the process as a single product or be present in a fatty acid mixture.

Advantageously, the substituents R^2 or R^3 in the general formulae I and II independently of one another are saturated or unsaturated C_2o-C_{22}-alkylcarbonyl; especially advantageously, are independently of one another unsaturated C_2o- or C_{22}-alkylcarbonyl with at least two double bonds.

The polyunsaturated fatty acids according to the present invention are, preferably, bound in membrane lipids and/or triacylglycerides, but may also occur in the organisms as free fatty acids or else bound in the form of other fatty acid esters. In this context, they may be present as "pure products" or else advantageously in the form of mixtures of various fatty acids or mixtures of different glycerides. The various fatty acids which are bound in the triacylglycerides can be derived from short-chain fatty acids with 4 to 6 C atoms, medium-chain fatty acids with 8 to 12 C atoms or long-chain fatty acids with

14 to 24 C atoms. In accordance with the method of the present invention, preferred are the long-chain fatty acids, especially the long chain PUFAs (LCPUFAs) of C_{20}- and/or C_{22}-fatty acids.

5 Preferred unsaturated fatty acids in the sense of the present invention are selected from the group consisting of ARA 20:4 (5,8,1 1,14), EPA 20:5 (5,8,1 1,14,17), and DHA 22:6 (4,7,10,13,16,19). The ARA, EPA and/or DHA produced in the process may be, as described above, in the form of fatty acid derivatives, for example sphingolipids, phosphoglycerides, lipids, glycolipids, phospholipids, monoacylglycerol, diacylglycerol,
10 triacylglycerol or other fatty acid esters. The ARA, EPA and/or DHA and other polyunsaturated fatty acids which are present can be liberated for example *via* treatment with alkali, for example aqueous KOH or NaOH, or acid hydrolysis, advantageously in the presence of an alcohol such as methanol or ethanol, or *via* enzymatic cleavage, and isolated *via,* for example, phase separation and subsequent
15 acidification *via,* for example, H_2SO_4. The fatty acids can also be liberated directly without the above-described processing step.

The term "desaturase target molecule", preferably, encompasses substrates of the polypeptides of the invention. A particular preferred target molecule is oleic acid, li-
20 noleic acid, γ-linolenic acid, α-linolenic acid, dihomo-γ-linolenic acid, stearidonic acid, eicosatetraenoic acid (n-3), arachidonic acid, eicosapentaenoic acid and docosapentaenoic acid.

The present invention provides for a method for the manufacture of oil comprising the
25 steps of the aforementioned methods and the further step of formulating or isolating oil comprising the said unsaturated fatty acid.

The present invention also relates to a method of producing a host cell, plant or plant seed capable of generating an unsaturated fatty acid comprising introducing into said
30 host cell, plant or plant seed the nucleic acid molecule of the present invention or the vector of the present invention.

The present invention includes oil produced by the plant or plant seed of the invention, obtainable by the method of the invention or obtainable by a host cell, plant or plant
35 seed produced by the aforementioned method.

The term "oil" refers to a fatty acid mixture comprising unsaturated or saturated, preferably esterified, fatty acid(s). The oil is preferably high in polyunsaturated free or, ad-

vantageously, esterified fatty acid(s), in particular the preferred LCPUFAs referred to herein above. The amount of unsaturated esterified fatty acids preferably amounts to approximately 30%, a content of 50% is more preferred, a content of 60%, 70%, 80% or more is even more preferred. For the analysis, the fatty acid content can, for exam-
5 pie, be determined by GC after converting the fatty acids into the methyl esters by transesterification. The oil can comprise various other saturated or unsaturated fatty acids, for example calendulic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid and the like. The content of the various fatty acids in the oil or fat can vary, in particular depending on the starting organism. The oil, however, shall have a non-naturally
10 occurring composition with respect to the unsaturated fatty acid composition and content. Moreover, the oil of the invention may comprise other molecular species as well. Specifically, it may comprise minor impurities of the nucleic acid molecules of the invention. Such impurities, however, can be detected only by highly sensitive techniques such as PCR.
15

The present invention also includes a method for the manufacture of a medicament comprising the steps of the methods of the invention and the further step of formulating medicament comprising the said unsaturated fatty acid.

20 The term "medicament" is used herein interchangeably with the term "pharmaceutical composition" explained in detail below. The term "medicament" or "pharmaceutical composition" as used herein comprises the compounds of the present invention and optionally one or more pharmaceutically acceptable carrier. The compounds of the present invention can be formulated as pharmaceutically acceptable salts. Acceptable
25 salts comprise acetate, methylester, HCl, sulfate, chloride and the like. The pharmaceutical compositions are, preferably, administered topically or systemically. Suitable routes of administration conventionally used for drug administration are oral, intravenous, or parenteral administration as well as inhalation. However, depending on the nature and mode of action of a compound, the pharmaceutical compositions may be
30 administered by other routes as well. For example, polynucleotide compounds may be administered in a gene therapy approach by using viral vectors or viruses or liposomes.

Moreover, the compounds can be administered in combination with other drugs either in a common pharmaceutical composition or as separated pharmaceutical composi-
35 tions wherein said separated pharmaceutical compositions may be provided in form of a kit of parts.

The compounds are, preferably, administered in conventional dosage forms prepared by combining the drugs with standard pharmaceutical carriers according to conventional procedures. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables.

The carrier(s) must be acceptable in the sense of being compatible with the other ingredients of the formulation and being not deleterious to the recipient thereof. The pharmaceutical carrier employed may be, for example, either a solid, a gel or a liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are phosphate buffered saline solution, syrup, oil such as peanut oil and olive oil, water, emulsions, various types of wetting agents, sterile solutions and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl mono-stearate or glyceryl distearate alone or with a wax. Said suitable carriers comprise those mentioned above and others well known in the art, see, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania.

The diluent(s) is/are selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants, or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like.

A therapeutically effective dose refers to an amount of the compounds to be used in a pharmaceutical composition of the present invention which prevents, ameliorates or treats the symptoms accompanying a disease or condition referred to in this specification. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50.

The dosage regimen will be determined by the attending physician and other clinical factors; preferably in accordance with any one of the above described methods. As is well known in the medical arts, dosages for any one patient depends upon many fac-

tors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Progress can be monitored by periodic assessment. A typical dose can be, for example, in the range of 1 to 1000 µg; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. Generally, the regimen as a regular administration of the pharmaceutical composition should be in the range of 1 µg to 10 mg units per day. If the regimen is a continuous infusion, it should also be in the range of 1 µg to 10 mg units per kilogram of body weight per minute, respectively. Progress can be monitored by periodic assessment. However, depending on the subject and the mode of administration, the quantity of substance administration may vary over a wide range.

The pharmaceutical compositions and formulations referred to herein are administered at least once in order to treat or ameliorate or prevent a disease or condition recited in this specification. However, the said pharmaceutical compositions may be administered more than one time, for example from one to four times daily up to a non-limited number of days.

Specific pharmaceutical compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound referred to herein above in admixture or otherwise associated with a pharmaceutically acceptable carrier or diluent. For making those specific pharmaceutical compositions, the active compound^) will usually be mixed with a carrier or the diluent, or enclosed or encapsulated in a capsule, sachet, cachet, paper or other suitable containers or vehicles. The resulting formulations are to be adapted to the mode of administration, i.e. in the forms of tablets, capsules, suppositories, solutions, suspensions or the like. Dosage recommendations shall be indicated in the prescribers or users instructions in order to anticipate dose adjustments depending on the considered recipient.

For cosmetic applications, the compounds referred to herein as pharmaceutically active ingredients of the medicament can be formulated as a hair tonic, a hair restorer composition, a shampoo, a powder, a jelly, a hair rinse, an ointment, a hair lotion, a paste, a hair cream, a hair spray and/or a hair aerosol.

The present invention relatest to a medicament comprising the polynucleotide, the vector, the host cell, the polypeptide, the plant or plant seed or the oil of the present invention.

Moreover, the present invention relates to the use of the the polynucleotide, the vector, the host cell, the polypeptide, the plant or plant seed or the oil of the invention for the manufature of animal feed, a dietary supplement, or food.

Finally, the present invention relates to a cell comprising a nucleic acid molecule selected from the group consisting of:

a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 31, 33, 35, 37, 102, 104, 106, 108, 110, 112, 135, 137 or 139, wherein the nucleic acid molecule is disrupted by at least one technique selected from the group consisting of a point mutation, a truncation, an inversion, a deletion, an addition, a substitution and homologous recombination;

b) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 31, 33, 35, 37, 102, 104, 106, 108, 110, 112, 135, 137 or 139, wherein the nucleic acid molecule comprises one or more nucleic acid modifications as compared to the sequence set forth in SEQ ID NO: 1, 3, 5, 7, 31, 33, 35, 37, 102, 104, 106, 108, 110, 112, 135, 137 or 139, wherein the modification is selected from the group consisting of a point mutation, a truncation, an inversion, a deletion, an addition and a substitution; and

c) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 31, 33, 35, 37, 102, 104, 106, 108, 110, 112, 135, 137 or 139, wherein the regulatory region of the nucleic acid molecule is modified relative to the wild-type regulatory region of the molecule by at least one technique selected from the group consisting of a point mutation, a truncation, an inversion, a deletion, an addition, a substitution and homologous recombination.

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

FIGURES

FIGURE 1 shows various synthetic pathways for the biosynthesis of co-6 and co-3 fatty acids.

FIGURE 2 shows the alignment of SEQ ID NOS: 2, 4, 6, 8, 32, 34, 36 and 38 based on the ClustalW algorithm.

FIGURE 3 shows gas chromatography traces demonstrating the conversion of oleic acid (18:1 Δ9) into linoleic acid (18:2Δ9,12) proving the Δ12-desaturase activity of d12Des(Dt) (B), d12Des(Dn) (C) and d12Des(Sn) (D). In (A) the vector control is shown without any d12-desaturase activity.

FIGURE 4 shows gas chromatography traces demonstrating the conversion of linoleic acid (18:2Δ9,12) to α-linolenic acid (18:3Δ9,12,15) proving the Δ15-desaturase activity of d15Des(Cyh) (A). In (B) the absence of a Δ12-desaturase activity for d15Des(Cyh) is shown.

FIGURE 5 shows the construction of binary vectors Napin-A, Napin-3, Napin-4, Napin-5 for plant transformation .

FIGURE 6 shows gas chromatography traces of seed oil from *Brassica napus* lines transformed with construct LJB1 139 producing high levels of EPA.

EXAMPLES

The invention will now be described in greater detail with reference to the following Examples which, however, shall not be construed whatsoever to limit the scope of this invention.

Example 1: Cloning of desaturases from Drechsler tritici-repentis, Cylindorcarpon heteronema, Diplodia natalensis, Stagonospora nodorum, Microdochium nivalae and Periplaneta americana

Materials and Methods.

Growth and harvesting of *Drechsler tritici-repentis, Cylindorcarpon heteronema, Diplodia natalensis, Stagonospora nodorum* and *Microdochium nivalae*

The fungi were obtained from DSMZ (German collection of microorgamisms and cells, Braunschweig) and grown on plates under following conditions:

Drechsler tritici-repentis	2% malt, 0,8% agar	18-22^{0}C
Cylindorcarpon heteronema	2% Lima-Bean, 0,8% agar	24^{0}C

Diplodia natalensis	2% malt, 0,8% agar	22^{0}C
Stagonospora nodorum	CzapekDox-V8-Agar with 15g sucrose	18^{0}C

RNA isolation was done according to manufactures protocol using the RNAeasy Kit from Qiagen.

Growth and harvesting of P. americana.

Periplaneta americana were cultivated at 25-30°C at 70-80% humidity on garden soil. As feed dog food added with fresh vegetables was used. RNA isolation was done as described in EP0464553.

Nucleic acid manipulation and PCR-based cloning.

RNA isolation was done as described above. Transcripts were analyzed by reverse transcriptase PCR (RT-PCR). First strand cDNA was synthesised from total RNA using the SMART RACE cDNA Amplification kit (BD-Clontech, Basingstoke, UK) according to the manufacture's instructions. Single -stranded cDNAs were amplified with following primers.

Used primer for cloning of *Drechsler tritici-repentis, Cylindorcarpon heteronema, Diplodia natalensis, Stagonospora nodorum* and *Microdochium nivalae* desaturases:

Zan 348 (F)	**SEQ ID** NO: 88	**ACI GGI BTI TGG RTI BTI GSI CAY**
Zan 349 (F)	**SEQ ID** NO: 89	**SAI GAR YTI KBI GGI TGG SMI**
Zan 350 (R)	**SEQ ID** NO: 90	**IGT DAT IRV IAC IAR CCA RTG**
Zan 351 (R)	**SEQ ID** NO: 91	**RTG IDW IYS IAY DAT ICC RTG**

Used primer for cloning of *Periplaneta americana* desaturases:

5

SenO12 (F):	SEQ ID NO: 92	GAC CAY CGH VWG CAY CAY
SenO13 (F):	SEQ ID NO: 93	GAR ACX RAY GSN GAY CC
SenO14 (F):	SEQ ID NO: 94	GAR ACX RAY GSN GAY CCY XXX AAY KG
SenO15 (R):	SEQ ID NO: 95	GYY YKG TAR TCC CAK GGG WA
SenO16 (R):	SEQ ID NO: 96	TCC CAX GGR WAX RYR TGR TGR WAR TTG TG
SenO21d (F):	SEQ ID NO: 97	GAY CCI CAY AAY GCI AAR MGI GGI
SenO22d (F):	SEQ ID NO: 98	IGI GGI TTY TTY TTY WSI CAY
SenO23d (F):	SEQ ID NO: 99	TTY TTY WSI CAY GTI GGI TGG
SenO24d (R):	SEQ ID NO: 100	RTG IGC IGC ISW RTT IAC IAR CCA
SenO25d (R):	SEQ ID NO: 101	YTT RTC RTA IGG ICW ISW ICC CCA

Degenerated primers are in IUPAC standard nomenclature.

PCR amplification was done in the following way: The reactions were heated to 95 C for 2 min followed by 30 cycles at 94 C for 30 s, 30 s at temperatures ranging from 55
10 to 72 according to the primer design and 72 C for 2 min, then a single step at 72 C for 10 min. PCR amplification products were cloned into TOPO vector (Invitrogen) and verified by sequencing.
From the above described set of primer only the primer pairs Zan348-350 and SenO14-015 delivered fragments with sequence homologies to desaturases.
15 By using the Smart-RACE Kit (BD-Clontech, Basingstoke, UK) according to manufactures protocol 5' and 3' regions of the DNA pieces were further exploited.
Full-length sequences were obtained with the primer pairs described in Table 1.

Table 1: Amplification of full-length desaturase genes

Gene name	SEQ ID	Primer pair	Fragment length	ORF length
D12Des(Dt)	1	5'- ctgagagaacatgacgacgac SEQ ID39 5'- gtcgcttactcgttgtcactctc SEQ ID 40	1445	1344
D15Des(Cyh)	3	5'- gatactaagccaccaacatgg SEQ ID 41 5'- cgttctacgagccctctatttc SEQ ID 42	1259	1227
D12Des(Dn)	5	5'- caaccacccatcatggccac SEQ ID 43 5'- atcgcttcatgcgtcattgtc SEQ ID 44	1501	1482
D12Des(Sn)	7	5'- caccatcatggccaccacaac SEQ ID 45 5'- agctctactcgttgtcggactc SEQ ID 46	1496	1485
D12Des(Mn)	31	5'- atgggccatgagtgtggacac SEQ ID 47 5'- ctatttggccatcttggtaggggg SEQ ID 48	975	975
D15Des(Mn)	33	5'- atgattgcgaccacccagac SEQ ID 49 5'- ctaaaggtccttgcggggtgcg SEQ ID 50	1209	1209
dXDes(Pa)_1	35	5'- atggctccgaacattacaagttc SEQ ID 51 5'- ctacttgagcttcttgttgatg SEQ ID 52	1074	1074
dXDes(Pa)_2	37	5'- atggcccctaatataactagtac SEQ ID 53 5'- ttaatctttcttgttgattatttg SEQ ID 54	1071	1071

From the full-length fragments the ORF sequences and the amino acid sequences de-
5 duced (Table 2).

Table 2: Amino acid sequences from desaturase genes

Gene name	Amino acid sequence length	SEQ ID
D12Des(Dt)	447	2
D15Des(Cyh)	408	4
D12Des(Dn)	493	6
D12Des(Sn)	494	8
D12Des(Mn)	324	32
D15Des(Mn)	402	34
dXDes(Pa)_1	357	36
dXDes(Pa)_2	356	38

PCR fragments for full-length sequences were cloned into pCR-bluntII-TOPO vector (Invitrogen) according to manufactures protocol and verified by sequencing.

Cloning of Yeast expression vectors

5 For expression and characterization in yeast ORF sequences of new desaturase genes were cloned into the vector pYES2-TOPO (Invitrogen) by amplifying the ORF sequence with primers adding a Kozak sequence at the 5'-end (Table 3). The same PCR protocol as described above was used. The vectors described in Table 4 were generated and used for functional characterization of the new desaturase genes.

10

Table 3: Primer pairs for amplification of ORF sequences from new desaturases

Gene name	Primer pair	SEQ ID
D12Des(Dt)	5'- accatggacgagcagcctgccgtc 5'- ttactcgttgtcactctcag	55 56
D15Des(Cyh)	5'- accatggcggtccgacaacgcacc 5'- ctatttcgcccacctcatcgc	57 58
D12Des(Dn)	5'- accatggccaccaccgccatggctc 5'- tcatgcgtcattgtcgctgtcg	59 60
D12Des(Sn)	5'- accatggccaccacaactgcccgcgc 5'- ctactcgttgtcggactcagggcc	61 62
D12Des(Mn)	5'- accatgggccatgagtgtggacacc 5'- ctatttggccatcttggtaggggg	63 64
D15Des(Mn)	5'- accatgattgcgaccacccagac 5'- ctaaaggtccttgcggggtgcg	65 66
dXDes(Pa)_1	5'- accatggctccgaacattacaag 5'- ctacttgagcttcttgttgatg	67 68
dXDes(Pa)_2	5'- accatggcccctaatataactag 5'- ttaatctttcttgttgattatttg	69 70

Table 4: Yeast expression vectors

15

Gene name	ORF SEQ ID NO:	Expression vector	Short name of expression vector
D12Des(Dt)	126	pYES2-bluntII-TOPO-d12Des(Dt)	pYES-Dt
D15Des(Cyh)	127	pYES2-bluntII-TOPO-	pYES-Cyh

		d12Des(Cyh)	
D12Des(Dn)	128	pYES2-bluntII-TOPO-d12Des(Dn)	pYES-Dn
D12Des(Sn)	129	pYES2-bluntII-TOPO-d12Des(Sn)	pYES-Sn
D12Des(Mn)	31	pYES2-bluntII-TOPO-d12Des(Mn)	pYES-12Mn
D15Des(Mn)	130	pYES2-bluntII-TOPO-d12Des(Mn)	pYES-15Mn
dXDes(Pa)_1	131	pYES2-bluntII-TOPO-d12Des(Pa)	pYES-Pa1
dXDes(Pa)_2	132	pYES2-bluntII-TOPO-d12Des(Pa)	pYES-Pa2

Functional characterisation in Saccharomyces cerevisiae.

S.cerevisiae strain INVSC (Invitrogen) was transformed with the the constructs described in Table 4 or the empty vector (pYES2-bluntII-TOPO) as a control. Transformed cells were grown in a minimal medium containing raffinose and induced with 2% galactose. After 48h of growth total yeast fatty acids were extracted and the resulting FAMEs analysed by GC.

GC analysis of yeast transformed with pYES-Dt, pYES-Dn and pYES-Sn (Figure 3) revealed that an additional fatty acid was produced, which was identified as linoleic acid indicating that the gene we had cloned encoded a delta12 desaturase. Yeast cells expressing the delta12 desaturases are capable of recognizing $C18:1^{\Delta 9}$ substrate. When the fatty acid $18:2^{\Delta 9, 12}$ was added to the yeast medium, further differences between each desaturase could be identified. Yet further, another fatty acid from yeast lipid composition $16:1^{\Delta 7}$ is also converted to $16:2^{\Delta 7,10}$ in yeast transformed with pYES-Dn and pYES-Sn, whereas cells with pYES-Dt do not show this activity.

The different activities as indicated in Table 5 demonstrate the functional activity of the new desaturases. Based on the major activity they are named as d12-desaturase. Further the other activities are proof that the newly found desaturases are distinct from each other and show new activities for this class of enzymes which has not been demonstrated before. Especially the high activity of the d12Des(Dn) was unexpected and to our knowledge not reported for any known d12-desaturase.

For d15Des(Cyh) only activity with $18:2^{\Delta 9,12}$ could be shown, converting $18:2^{\Delta 9,12}$ into

$18:3^{\Delta 9,12,15}$. Therefore the d15Des(Cyh) has delta 15-desaturase activity and was named therefore Δ15-desaturase.

The percentage conversion is calculated for example of $18:1^{\Delta 9}$ to $18:2^{\Delta 9,12}$ by following
5 equation:

$$\% \text{ conversion} = \frac{[18:2^{\Delta 9,12}]}{[18:2^{\Delta 9,12}] + [18:1^{\Delta 9}]} \times 100$$

Table 5: Functional characterization of newly found desaturases

10

Gene name	Conversion rate of 18:1 to 18:2 in %	Conversion rate of 16:1 to 16:2 in %	Conversion rate of 18:2 to 18:3 in %
D12Des(Dt)	27,5	-	6,3
D15Des(Cyh)	-	-	14,9
D12Des(Dn)	76,3	49,3	-
D12Des(Sn)	52,5	31,2	-

Example 2: Production of constructs for expression in plants

15 For the expression of new desaturase genes in plant, binary plasmids for *Agrobacterium* transformation are designed.
Further, constructs for the multiple expression of the whole pathway towards eicosapentaenoic acid were constructed. These constructs utilize a number of enzymatic activities, namely d12-desaturase (conversion of oleic to linoleic acid), d15-desaturase
20 (conversion of linoleic to linolenic acid), d6-desaturase (conversion of linoleic and linolenic acid to γ-linolenic and stearidonic acid, respectively), d6-elongase (conversion of γ-linolenic and stearidonic acid to dihomogammalinolenic and eicosatetraenoic acid), d5-desaturase (conversion of dihomogammalinolenic and eicosatetraenoic acid to ARA and EPA), ω3-desaturase (conversion of ARA to EPA). Such a construct was assem-
25 bled by using standard molecular biology methods known to a person skilled in the art. The sequence of the complete binary vector (LJB1 139) is listed in SEQ ID NO: 71. The vector elements are described in Table 6.

Table 6: Functional elements of binary vector LJB1 139 depicted from SEQ ID NO: 68.

Element	**Description**	**Position from start**	**SEQ ID NO:**
D6Elo(Tp)	D6-elongase *Thalassiosira pseudonana*	1780-2598	11
D6Des(Ot)	D6-desaturase *Ostreococcus tauri*	3756-5126	19
D12Des(Ps)	D12-desaturase *Phytophtora sojae*	6484-7680	21
O3Des(Pi)	ω3-desaturase *Phytophtora infestans*	8873-9958	17
D5Des(Tc)	D5-desaturase *Thraustochytrium ssp.*	11622-12941	13
D15Des(At)	D15-desaturase *Arabidopsis thaliana*	17834-18994	23
BnFae1	Promoter BnFAE1	347-1776	76
t-bnfae1	Terminator bnfae1	2612-3011	77
VfUSP	Promoter VfUSP	3061-3744	78
t-camv	Terminator CaMV-35S	5179-5394	79
BnACP	Promoter BnACP	5466-6465	80
t-bnacp	Terminator BnACP	7694-8075	81
BnNapin	Promoter BnNApin	8206-8869	82
tE9	Terminator tE9	9959-10516	83
LuCon	Promoter LuCon	10545-11583	84
tOCS	Terminator tOCS	12993-13184	85
BnNapin	Promoter BnNApin	13263-13926	82
tE9	Terminator tE9	15348-15905	83
VfSBP	Promoter VfSBP	16827-17824	86
tCatpA	Terminator tCatpA	19022-19256	87

5

Further other binary vectors were constructed according to following experimental steps:

For building the constructs Napin-3, Napin-4 and Napin-5, a triple cassette containing three napin promoters, three different multiple cloning site linkers and three octopine
10 synthase (OCS) terminators was constructed in the plasmid pUC19 (Genbank M77789). A three-gene construct was built by inserting PiΔ6, a Δ6 desaturase gene from *Pythium irregulare* (Hong *et al.,* 2002 High-level production of γ-linolenic acid in *Brassica juncea* using a Δ6 desaturase from *Pythium irregulare.* Plant Physiol. **129,** 354-362) (SEQ ID NO: 9), TcΔ5, a Δ5 desaturase gene from *Thraustochytrium sp.*
15 ATCC 26185 (Qiu *et al.,* 2001 Identification of a Δ4 fatty acid desaturase from *Thraus-*

tochytrium sp. involved in the biosynthesis of docosahexanoic acid by heterologous expression in *Sacchcaromyces cerevisiae* and *Brassica juncea.* J. Biol. Chem. **276,** 31561-31566) (SEQ ID NO: 13) and TpElo, an elongase gene from *Thalassiosira pseudonana* (Meyer *et ai,* 2004 Novel fatty acid elongases and their use for the recon-
5 stitution of docosahexaenoic acid biosynthesis. J. Lipid Res. 45, 1899-1909) (SEQ ID NO: 11) into this cassette. For the four gene construct Napin-4, an Xhol/Sall fragment containing the desaturase gene CpDesX from *Claviceps purpurea* (Meesapyodsuk *et ai,* 2007 Primary structure, regioselectivity, and evoloution of the membrane-bound fatty acid desaturases of *Claviceps purpurea.* J. Biol. Chem. **282,** 20191-20199) (SEQ
10 ID NO: 25) linked to a napin promoter and OCS terminator was removed from a single-gene construct and subcloned into the triple gene construct. For the five gene construct Napin-5, the same approach was used to add an ω3 desaturase gene from *Pythium irregulare* (Pi-ω3) (SEQ ID NO: 17). Finally these 3-, 4-, and 5-gene constructs were removed from pUC19 by digestion with Ascl, and cloned into the binary vector pSUN2
15 (Figure 5). The Napin-A construct , which contained the same a Δ5 and Δ6 desaturases described above, plus a Δ6 elongase from *Thraustochytrium* sp. (SEQ ID NO: 15), an ω3 desaturase gene from *Phytophtera infestans* (SEQ ID NO: 17), and a Δ12 desaturase from *Calendula officianalis* (SEQ ID NO: 72), was constructed as described in Wu *et al.* (2005) Stepwise engineering to produce high yields of very long-chain polyun-
20 saturated fatty acids in plants. Nat. Biotechnol. 23, 1013-1017). Napin-A represents a five-gene construct with each gene under the control of napin promoter.

All binary vectors were transferred into *Agrobacterium tumefaciens* strain GV3101 (pMP90) by electroporation.

The resulting *Agrobacterium* strains were subsequently used for the production of
25 transgenic plants (Deblaere et al. 1984, Nucl. Acids. Res. 13: 4777-4788).

Example 3: Production of transgenic plants

30 a) Generation of transgenic rape seed plants (amended protocol according to Moloney et al. 1992, Plant Cell Reports, 8:238-242)

For the transformation of rapeseed plants a 1:50 dilution of an overnight culture of positive transformed acrobacteria colonies grown in Murashige-Skoog Medium (Murashige
35 and Skoog 1962 Physiol. Plant. 15, 473) supplemented by 3% saccharose (3MS-Medium) was used. Petiols or Hypocotyledones of sterial rapeseed plants were incubated in a petri dish with a 1:50 acrobacterial dilusion for 5-10 minutes. This was followed by a tree day co-incubation in darkness at 25°C on 3MS-Medium with 0.8%

bacto-Agar. After three days the culture was put on to 16 hours light/8 hours darkness weekly on MS-medium containing 500mg/l Claforan (Cefotaxime-Natrium), 50mg/l Kanamycine, 20 mikroM Benzylaminopurin (BAP) and 1,6g/l Glucose. Growing sprouts were transferred to MS-Medium containing 2% saccharose, 250mg/l Claforan and 0.8%Bacto-Agar. Even after three weeks no root formation was observed, a growth hormone 2-Indolbutyl acid was added to the medium for enhancing root formation.

Regenerated sprouts have been obtained on 2MS-Medium with Kanamycine and Claforan and were transferred to the green house for sprouting. After flowering, the mature seeds were harvested and analysed for expression of the genes via lipid analysis as described in Qui et al. 2001 , J. Biol. Chem. 276, 31561-31566.

b) Generation of transgenic *Brassica carinata* plants (amended protocol according to Moloney et al. 1992, Plant Cell Reports, 8:238-242)

Seeds of *B. carinata* lines C90-1 163 (high erucic acid line) and 10H3 (zero-erucic acid line) were used for transformation. The protocol described by Moloney et al. (1992)was used for *B. carinata* transformation. Cotyledon petioles from 5 to 6 days old seedlings were excised and inoculated with *A. tumefaciens* strain GV3101 containing the desired gene construct and co-cultured for two days at 22^{0}C before being transferred to the regeneration medium containing 25mg/l_ kanamycin.

c) Production of transgenic flax plants

The production of transgenic flax plants can be carried out according to the method of Bell et al., 1999, In Vitro Cell. Dev. Biol. Plant 35(6):456-465 using particle bombardment. Acrobacterial transformation could be carried out according to Mlynarova et al. (1994), Plant Cell Report 13: 282-285.

All obtained putative transgenic plants for all experiments were validated by PCR analysis.

Example 4: Lipid Extraction

Lipids can be extracted as described in the standard literature including Ullman, Encyclopedia of Industrial Chemistry, Bd. A2, S. 89-90 und S. 443-613, VCH: Weinheim (1985); Fallon, A., et al., (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, Bd. 17; Rehm et al. (1993) Bio-

technology, Bd. 3, Kapitel III: "Product recovery and purification", S. 469-714, VCH: Weinheim; Belter, P.A., et al. (1988) Bioseparations: downstream processing for Biotechnology, John Wiley and Sons; Kennedy, J.F., und Cabral, J.M.S. (1992) Recovery processes for biological Materials, John Wiley and Sons; Shaeiwitz, J.A., und Henry, J.D. (1988) Biochemical Separations, in: Ullmann's Encyclopedia of Industrial Chemistry, Bd. B3; Kapitel 11, S. 1-27, VCH: Weinheim; und Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications.

Alternatively, extraction will be carried out as described in Cahoon et al. (1999) Proc. Natl. Acad. Sci. USA 96 (22): 12935-12940, und Browse et al. (1986) Analytic Biochemistry 152:141-145. Quantitative and qualitative analysis of lipids or fatty acids are described in Christie, William W., Advances in Lipid Methodology, Ayr/Scotland: Oily Press (Oily Press Lipid Library; 2); Christie, William W., Gas Chromatography and Lipids. A Practical Guide - Ayr, Scotland: Oily Press, 1989, Repr. 1992, IX, 307 S. (Oily Press Lipid Library, 1); "Progress in Lipid Research, Oxford: Pergamon Press, 1 (1952) - 16 (1977) u.d.T.: Progress in the Chemistry of Fats and Other Lipids CODEN.

Based on the analysed lipids, the expression of the introduced genes (desaturases and elongases) can be determined since the lipid pattern of successfully transformed plant seeds will differ from the pattern of control plant seeds. Fatty acid analyses of seeds were performed by GC as described by Qiu *et al.* J. Biol. Chem. 276, 31561-31566.

Example 5: Production of novel plant oils

For the construct LJB1139 (SEQ ID NO: 71) transgenic *Brassica napus* plants were obtained. Fatty acid analysis of seeds revealed that the expected new fatty acids were produced (Figure 6). Oleic and linoleic acid were reduced compared to non-transgenic *Brassica napus* oil to 11,7% and 2,3%, respectively. GLA (24,4%) and SDA (6,8%) are newly produced fatty acids. Further high levels of EPA could be achieved (up to 21,7% EPA). The oil constitutes very beneficial fatty acids for human consumption and feed. Other gene combinations producing beneficial fatty acid compositions are with removing SEQ ID NO: 23 in LJB1139 and adding SEQ ID NO: 25, SEQ ID NO: 27 or SEQ ID NO: 29, respectively. Further removing SEQ ID NO: 11 and adding SEQ ID NO: 133 results in another novel fatty acid spectrum

For the high-erucic acid *B. carinata* line transgenic plants carrying the Napin-A construct were obtained. The plants produced the expected new fatty acids (Table 7). In

these plants, GLA and SDA averaged 18.2% and 2.9% of total fatty acids in seed, respectively. The amount of ARA ranged from 0.4-4.3% (average: 2.8%), while EPA reached an average level of 9.3%, with the highest value observed being 13.7%. The average amount of DPA was 1.4%. BothD 6 and D 5 desaturases functioned very well,
5 with conversion levels of 81.5% and 87.1 %, respectively, while theD6 elongase showed a conversion level of 42.5%.

Table 7: Gas chromatographic analysis of seed oil from transgenic high-erucic acid *B. carinata* lines.
10

	***B. carinata* high erucic line**	
Fatty acid	**Control**	**Transgenic**
16:0	5.3±0.7	6.3±1.2
18:0	1.2±0.2	1.5±0.4
18:1n-9 (OA)	7.3±0.7	5.5±3.9
18:1n-11	1.5±0.2	1.7±0.2
18:2n-9	0.0	0.2±0.3
18:2n-6 (LA)	16.5±0.9	5.8±4.2
18:3n-6 (GLA)	0.0	18.2±4.2
		(8.9-23.3)
18:3n-3 (ALA)	16.0±1.3	2.4±2.1
18:4n-3 (SDA)	0.0	2.9±0.6
		(2.8-4.5)
20:1n-9	6.2±1.0	4.5±0.6
20:1n-7	1.4±0.1	1.7±0.3
20:3n-6 (DGLA	0.0	0.9±0.2
20:4n-6 (AA)	0.0	2.8±1.5
		(0.4-4.3)
20:4n-3 (ETA)	0.0	1.0±0.4
20:5n-3 (EPA)	0.0	9.3±4.4
		(1.4-13.7)
22:5n-3 (DPA)	0.0	1.4±0.9
Erucic	36.1±2.4	25.7±3.6
Other	8.5±1.5	8.4±1.8

For the low-erucic acid *Brassica carinata* line transgenic plants carrying the Napin-A construct were obtained, and the seeds contained the expected novel fatty acids at
15 various levels (Table 8). The amounts of GLA and SDA averaged 29.6% (range: 27.7-31.7%) and 6.2% (range: 3.9-8.1%) of total fatty acids, respectively. While ARA had an average level of 6.5% (range: 6.1-6.9%), EPA reached 25.0% in an individual seed with an average of 20.2%. DPA content averaged 2.5%. The aggregate amount of novel fatty acids reached 69.2% of total fatty acids. Both the D6 and D5 desaturases func-
20 tioned very well, with conversion levels of 91.2% and 87.7%, respectively. TheD6 elongase showed a conversion level of 47.9% in the low-erucic acid *B. carinata* line,

which was higher than was observed in either the high-erucic acid *B. carinata* line. Transgenic low-erucic acid *B. carinata* produced one of the highest levels of 22-carbon PUFAs obtained to date, and this was achieved without the use of an elongase specific for D5-desaturated products.

5

Table 8: Gas chromatographic analysis of seed oil from transgenic low-erucic acid *B. carinata* lines.

	B. carinata low erucic-acid	
Fatty acid	**Control**	**Transgenic**
16:0	7.1±0.6	5.8±0.5
18:0	1.7±0.3	2.1±0.6
18:1n-9 (OA)	18.9±8.0	4.1±3.3
18:1n-11	3.5±0.3	3.8±0.4
18:2n-9	0.0	0.4±0.8
18:2n-6 (LA)	44.1±3.6	4.4±1.2
18:3n-6 (GLA)	0.0	29.6±1.6
		(27.7-31.7)
18:3n-3 (ALA)	21.0±6.4	2.3±0.7
18:4n-3 (SDA)	0.0	6.2±1.7
	0.0	(3.9-8.1)
20:1n-9	0.0	0.0
20:1n-7	0.0	0.0
20:3n-6 (DGL	0.0	2.0±0.7
20:4n-6 (AA)	0.0	6.5±0.3
	0.0	(6.1-6.9)
20:4n-3 (ETA)	0.0	2.1±0.7
20:5n-3 (EPA)	0.0	20.2±4.5
	0.0	(15.3-25.0)
22:5n-3 (DPA)	0.0	2.5±0.7
Erucic	0.0	0.0
Other	3.8±0.7	8.1±0.5

10

The effects of two desaturases on EPA production in B. carinata

A minimal set of three genes, comprising aΔ5 desaturase, aΔ6 desaturase and aΔ6

15 elongase, is required for the synthesis of ARA and EPA from endogenous LA ($18:2^{\Delta 9,12}$) and ALA ($18:3^{\Delta 9,12,15}$). In order to quantify the contributions of the two novel desaturase genes, CpDesX and Pi-ω3, to EPA production in transgenic plants, we needed to know the base levels of ARA and EPA in plants carrying the minimal set of three trangenes. For this purpose, gene construct Napin-3 (Figure 5) containing a Δ6 desaturase from

20 *Pythium irregulare* (SEQ ID NO:9), aΔ5 desaturase from *Thraustochytrium* sp. (SEQ ID NO: 13), and aΔ6 elongase from *Thalassiosira pseudonana* (SEQ ID NO: 11) was in-

troduced into the high-erucic *B. carinata* line. In transgenic seeds of plants carrying Napin-3, GLA and SDA averaged 17.6% and 4.3% of total fatty acids, respectively (Table 9). The amount of ARA reached 12.2% (average: 8.4%), and EPA had an average level of 2.3% (range: 0.8%-3.5%). The Δ6 and Δ5 desaturases functioned very well, with
5 substrate conversion levels of 73.7% and 85.6%, respectively, while the conversion level of the Δ6 elongase was 36.3%. The total amount of novel co-6 fatty acids (GLA, DGLA and AA) represented 27.5% of seed fatty acids, while ω3 fatty acids (SDA, ETA and EPA) represented 6.9%, indicating that the ω6 pathway was operating more effectively than the ω3 pathway.
10

Table 9: Gas chromatographic analysis of seed oil from transgenic high-erucic acid *B. carinata* lines transformed with construct Napin-3.

Fatty acid	Wild type	Napin-3
16:0	5.3±0.7	7.2±1.5
18:0	1.2±0.2	1.4±0.4
18:1n-9 (OA)	7.3±0.7	8.2±3.8
18:1n-11	1.5±0.2	2.2±0.7
18:2n-9	0.0	2.0±1.6
18:2n-6 (LA)	16.5±0.9	3.6±6.9
18:3n-6 (GLA)	0.0	17.6±3.8
		(13.7-24.2)
18:3n-3 (ALA)	16.0±1.3	2.0±0.6
18:4n-3 (SDA)	0.0	4.3±1.3
		(2.4-6.3)
20:1n-9	6.2±1.0	4.8±1.3
20:1n-7	1.4±0.1	1.8±0.5
20:3n-6 (DGLA	0.0	1.5±0.9
20:4n-6 (AA)	0.0	8.4±2.5
		(4.3-12.2)
20:4n-3 (ETA)	0.0	0.3±0.4
20:5n-3 (EPA)	0.0	2.3±0.8
		(0.8-3.5)
Erucic	36.1±2.4	25.3±7.3
Other	8.5±1.5	7.2±1.0

15

In experiments in yeast, the 18-carbon ω3 desaturase gene CpDesX was able to convert LA to ALA, and GLA to SDA (Meesapyodsuk *et al.,* 2007). We felt that an increase in the initial ω3 substrate ALA and/or the conversion GLA to SDA might lead to higher EPA production. To determine if CpDesX could increase the level of ω3 fatty acids in
20 plants, this gene was added to the three-gene construct Napin-3, producing the four-gene construct Napin-4. Transgenic plants were obtained. The expression of CpDesX (SEQ ID NO: 25) substantially increased the levels of ω3 fatty acids; SDA reached 9.4% in an individual seed and the average level was 6.3%, while average EPA levels

increased to 4.2% from the 2.3% found in transgenic seeds carrying Napin-3 (Table 10). The total amounts of novel 006 and ω3 fatty acids averaged 19.0% and 10.9%, respectively, indicating an increase of flux through the ω3 pathway due to the activity of the CpDesX desaturase. This increase of ω3 fatty acids suggests that CpDesX can make an important contribution to EPA production in transgenic plants.

Table 10: Gas chromatographic analysis of seed oil from transgenic high-erucic acid *B. carinata* lines transformed with construct Napin-4.

Fatty acid	Wild type	Napin-4
16:0	5.3±0.7	6.6±1.5
18:0	1.2±0.2	1.3±0.4
18:1n-9 (OA)	7.3±0.7	7.8±2.5
18:1n-11	1.5±0.2	1.8±0.5
18:2n-9	0.0	0.9±0.6
18:2n-6 (LA)	16.5±0.9	4.0±3.0
18:3n-6 (GLA)	0.0	11.8±1.6
		(9.0-15.6)
18:3n-3 (ALA)	16.0±1.3	3.2±1.3
18:4n-3 (SDA)	0.0	6.3±2.0
		(4.1-9.4)
20:1n-9	6.2±1.0	4.6±1.1
20:1n-7	1.4±0.1	1.8±0.7
20:3n-6 (DGLA	0.0	0.7±0.3
20:4n-6 (AA)	0.0	6.5±1.9
		(1.8-9.5)
20:4n-3 (ETA)	0.0	0.4±0.2
20:5n-3 (EPA)	0.0	4.2±1.4
		(1.0-5.6)
Erucic	36.1±2.4	31.2±5.3
Other	8.5±1.5	6.9±2.4

To test the activity of the 20-carbon ω3 desaturase Pi-ω3 in converting ARA to EPA in plants, Pi-ω3 was added to the four gene construct Napin-4. Transgenic plants were obtained with producing containing the expected novel fatty acids at various levels (Table 11). Expression of the Pi-ω3 desaturase (SEQ ID NO: 17) results in the effective conversion of ARA to EPA. With the addition of this gene, EPA content increased substantially, from an average level of 4.2% in transgenic seeds carrying Napin-4 to an average level of 9.7% in transgenic seeds with Napin-5, with the highest value observed being 15.5%. The level of novel 006 and ω3 fatty acids averaged 13.0% and 16.5%, respectively. The high levels of EPA achieved in transgenic seeds carrying the Napin-5 construct can be attributed to the activities of the two novel desaturase genes (CpDesX and Pi-ω3).

Table 11: Gas chromatographic analysis of seed oil from transgenic high-erucic acid *B. carinata* lines transformed with construct Napin-5.

Fatty acid	Wild type	Napin-5
16:0	5.3±0.7	6.4±1.1
18:0	1.2±0.2	1.2±0.2
18:1n-9 (OA)	7.3±0.7	6.3±2.3
18:1n-11	1.5±0.2	1.6±0.2
18:2n-9	0.0	1.5±0.9
18:2n-6 (LA)	16.5±0.9	3.7±3.3
18:3n-6 (GLA)	0.0	11.1±2.4
		(4.8-15.3)
18:3n-3 (ALA)	16.0±1.3	3.4±2.9
18:4n-3 (SDA)	0.0	6.2±1.8
		(2.9-10.9)
20:1n-9	6.2±1.0	4.2±1.2
20:1n-7	1.4±0.1	2.1±0.4
20:3n-6 (DGLA	0.0	0.6±0.3
20:4n-6 (AA)	0.0	1.3±0.4
		(0.6-1.8)
20:4n-3 (ETA)	0.0	0.6±0.2
20:5n-3 (EPA)	0.0	9.7±3.4
		(1.5-15.5)
Erucic	36.1±2.4	31.1±3.8
Other	8.5±1.5	9.0±1.6

5

Example 6: Optimization of the production of novel oil products

As demonstrated in Example 5 by combination of different genes and regulatory elements it is possible to produce significant levels of novel fatty acids in the seed oil of different *Brassica* species. For further increase of special fatty acids like ARA, EPA and/or DHA or for the increase of overall production of novel fatty acids, additional genes might be required. Such additional genes could have functions in shuffling of different fatty acids from their respective pools of production (phospholipids, Acyl-CoA) or by esterifying the novel fatty acids to different molecules (diacylglycerol, lysophosphatic acid, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysophosphatidylinositol, lysophosphatidylserine, 1-acyl-phosphatidylcholine etc.). Acyltransferases utilize such activities as described above. In the past there were a number of approaches to isolate such activities to increase the production of novel fatty acids (for example WO2004/076617, WO2004/087902). Beside introducing new gene activities by transforming above described genes in combination with genes as described in Example 5 another another method is the modulation of endogenous

genes with acyltransferase activities by overexpression or down-regulation (antisense RNA, inverted RNA or miRNA technologies).

For *Brassica* species a number of acyltransferases could be identified, which have beneficial acyltransferase activity in terms of increased levels of ARA, EPA and/or DHA
5 or the overall production of novel fatty acids.

By using *Arabidopsis* homologues as described in Chen et al. (2007) FEBS Lett. 581 :551 1-5516 the cloning of a number of lysophosphatidyl-active acyltransferases could be isolated from *Brassica napus.* For isolation of the sequences standard PCR methods were used as described in Example 2 using following primer pairs (Table 12).
10 The PCR reactions resulted in 6 fragments coding for full-length ORF sequences coding for amino acid sequences (Table 13).

The ORF sequences shown in Table 13 can be used as described in Example 5 for the production of overexpressing, antisense, inverted RNA or miRNA constructs to up or downregulate the expression of the respective genes and thereby promoting changes
15 in the levels of novel fatty acids.

Table 12: Primer pairs for amplification of *Brassica napus* acyltransferases.

Name	Primer sequence	SEQ ID NO:
BnLPLAT1_A_40		
Fwd:	5'- atgatatcgatggacatgga	114
Rev:	5'- ttattcttctttacgtggctttg	115
BnLPLAT1_C_40		
Fwd:	5'- atgatatcgatggacatgaattc	116
Rev:	5'- ttattcctctttacgtggctttg	117
BnLPLAT2_C_50		
Fwd:	5'- atggaatcgctcgacatgag	118
Rev:	5'- ttattcttctttccgggtctttg	119
BnLPEAT1_A_50		
Fwd:	5'- atggaatcggagctaaagaa	120
Rev:	5'- ttattcttctttctgatggaaaac	121
BnLPEAT1_C_50		
Fwd:	5'- atggagtcggagctaaagga	122
Rev:	5'- tcattcttctttctgatggaaaac	123
BnLPEAT2_70		
Fwd:	5'- atggcgaatcctgatttgtc	124
Rev:	5'- ttatgttggggacaagatagg	125

Table 13: ORF sequences of *Brassica napus* acyltransferases.

Name	Activity	SEQ ID NO:
BnLPLATI _A_40	Lysophospholipid acyltransferase	102
BnLPLATI _C_40	Lysophospholipid acyltransferase	104
BnLPLAT2_C_50	Lysophospholipid acyltransferase	106
BnLPEAT1_A_50	Lysophosphatidyl ethanolamine acyltransferase	108
BnLPEAT1_C_50	Lysophosphatidyl ethanolamine acyltransferase	110
BnLPEAT2 J O	Lysophosphatidyl ethanolamine acyltransferase	112

5 Additional sequence variants of Brassica napus acyltransferases as described in Table 13 were identified. The additional sequences could be identified by a PCR approach as described in Example 6 using genomic DNA of the Brassica napus variety cv. Kumily as starting material. The sequence variants were functionally tested by overexpressing the respective ORF in a yeast KO mutant defective for the LPLAT activity (similar ex-
10 perimental yeast work as described in Example 1). The following sequence variants were identified:

BnLPLAT1_AA (ORF SEQ ID No. 137) is a variant of BnLPLAT1_A_40 (SEQ ID No.102);

BnLPLAT1_CC (ORF SEQ ID No. 139) is a variant of BnLPLAT1_C_40 (SEQ ID
15 No.104); and

BnLPLAT2_AA (ORF SEQ ID No. 135) is a variant of BnLPLAT2_C_50 (SEQ ID No. 106).

20

CLAIMS

1. A polynucleotide comprising a nucleic acid selected from the group consisting of:
a) a nucleic acid having a nucleic acid sequence as shown in any one of SEQ ID Nos: 1, 3, 5, 7, 31, 33, 35 or 37;
b) a nucleic acid encoding a polypeptide having an amino acid sequence as shown in any one of SEQ ID Nos: 2, 4, 6, 8, 32, 34, 36, or 38;
c) a nucleic acid which has a nucleic acid sequence being at least 60 % identical to the nucleic acid sequence as shown in any one of SEQ ID Nos: 1, 3, 5, 7, 31, 33, 35 or 37, wherein said nucleic acid encodes a polypeptide having desaturase activity;
d) a nucleic acid encoding a polypeptide having an amino acid sequence being at least 74.1% identical to the amino acid sequnce shown in any one of SEQ ID Nos: 2, 4, 6, 8, 32, 34, 36, or 38, wherein said nucleic acid encodes a polypeptide having desaturase activity,
e) a nucleic acid which hybridizes under stringent hybridization conditions to the nucleic acid of any one of a) to d), wherein said nucleic acid encodes a polypeptide having desaturase activity;
f) a nucleic acid encoding a fragment of a polypeptide encoded by the nucleic acid sequence of any one of a) to e) having desaturase activity; and.
g) a nucleic acid comprising at least 15 contigous nucleotides of the nucleic acid of any one of a) to f).

2. A vector comprising the polynucleotide of claim 1.

3. The vector of claim 2, which is an expression vector.

4. The vector of claim 3, wherein the said polynucleotide is under the control of a seed-specific promoter.

5. The vector of claim 4, wherein the seed-specific promoter is selected from the group consisting of Conlinin 1, Conlinin 2, napin, USP, LeB4, Arc, Fae, ACP, LuPXR, SBP and LuFad3.

6. A host cell comprising the polynucleotide of claim 1 or which is transformed with the expression vector of any one of claims 3 to 5.

7. The host cell of claim 6, wherein said cell is a plant cell.

8. The host cell of claim 7, wherein said plant cell is a cell obtained from an oilseed crop.

5 9. The host cell of claim 8, wherein the 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 10 seed, castor bean, lesquerella, tallow tree, sheanuts, tungnuts, kapok fruit, poppy seed, jojoba seeds and perilla.

10. The host cell of claim 6, wherein said cell is a microbial cell.

15 11. The host cell of claim 10, wherein the microbial cell is selected from the group consisting of *Candida, Cryptococcus, Lipomyces, Rhodosporidium, Yarrowia, Thraustochytrium, Pythium, Schizochytrium* and *Crythecodinium.*

12. A plant or plant seed comprising the polynucleotide of claim 1, the vector of any 20 one of claims 2 to 5 or the host cell of any one of claims 6 to 9.

13. A method of producing a polypeptide comprising culturing the host cell of any one of claims 6 to 11 in an appropriate culture medium to, thereby, produce the polypeptide encoded by the polynucleotide of claim 1.

25

14. A polypeptide encoded by the polynucleotide of claim 1.

15. A method for manufacturing an unsaturated fatty acid, comprising culturing the host cell of any one of claims 6 to 11 or the plant or plant seed of claim 12 such 30 that the unsaturated fatty acid is produced.

16. A method of modulating the production of a unsaturated fatty acid comprising culturing the host cell of any one of claims 6 to 11 or the plant or plant seed of claim 12, such that modulation of the production of the unsaturated fatty acid oc- 35 curs.

17. The method of claim 15 or 16, wherein said method further comprises the step of recovering the unsaturated fatty acid from said culture.

18. A method of manufacturing a unsaturated fatty acid comprising contacting a composition comprising at least one desaturase target molecule with at least one polypeptide of claim 14 under conditions such that the unsaturated fatty acid is produced.

19. The method of claim 21, wherein the desaturase target molecule is oleic acid, linoleic acid, γ-linolenic acid, α-linolenic acid, dihomo-γ-linolenic acid, stearidonic acid, eicosatetraenoic acid (n-3), arachidonic acid, eicosapentaenoic acid and docosapentaenoic acid.

20. The method of any one of claims 15 to 19, wherein the unsaturated fatty acid is selected from the group consisting of ARA 20:4 (5,8,11,14), EPA 20:5 (5,8,11,14,17), and DHA 22:6 (4,7,10,13,16,19).

21. A method for the manufacture of oil comprising the steps of the method of any one of claims 15 to 20 and the further step of formulating or isolating an oil comprising the said unsaturated fatty acid.

22. A method of producing a host cell, plant or plant seed capable of generating an unsaturated fatty acid comprising introducing into said host cell, plant or plant seed the polynucleotide of claim 1 or the vector of any one of claims 2 to 5.

23. An oil produced by the plant or plant seed of claim 12 or obtainable by the method of claim 20.

24. Use of the the polynucleotide of claim 1, the vector of any one of claims 2 to 5, the host cell of any one of claims 6 to 11, the plant or plant seed of claim 12 or the oil of claim 23 for the manufature of animal feed, a dietary supplement, or food.

25. A cell comprising a nucleic acid molecule selected from the group consisting of:
   a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 31, 33, 35 or 37, wherein the nucleic acid molecule is disrupted by at least one technique selected from the group consisting of a point mutation, a truncation, an inversion, a deletion, an addition, a substitution and homologous recombination;

b) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 31, 33, 35 or 37, wherein the nucleic acid molecule comprises one or more nucleic acid modifications as compared to the sequence set forth in SEQ ID NO:1 , 3, 5, 7 or 9, wherein the modification is selected from the group consisting of a point mutation, a truncation, an inversion, a deletion, an addition and a substitution; and

c) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 31, 33, 35 or 37, wherein the regulatory region of the nucleic acid molecule is modified relative to the wild-type regulatory region of the molecule by at least one technique selected from the group consisting of a point mutation, a truncation, an inversion, a deletion, an addition, a substitution and homologous recombination.

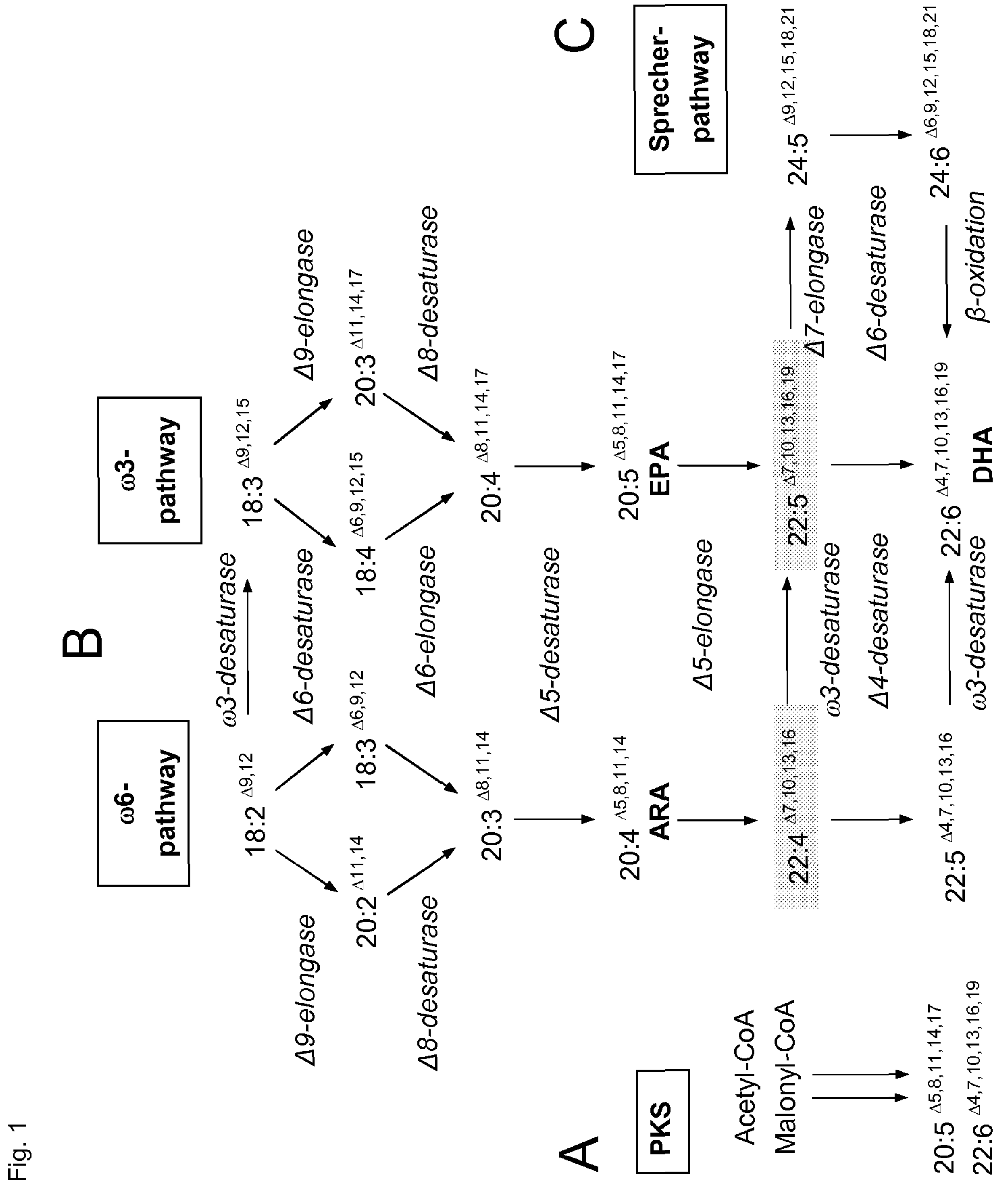


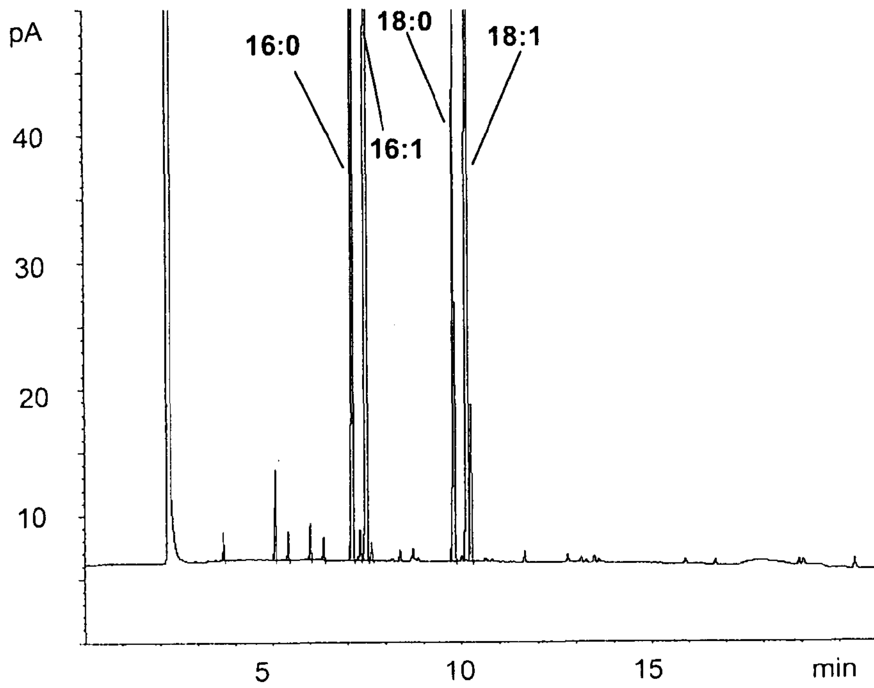
Fig. 1
A
PKS
Acetyl-CoA
Malonyl-CoA
20:5 Δ5,8,11,14,17
22:6 Δ4,7,10,13,16,19
B
ω6-pathway
ω3-pathway
18:2 Δ9,12
ω3-desaturase
18:3 Δ9,12,15
Δ9-elongase
Δ6-desaturase
Δ9-elongase
20:2 Δ11,14
18:3 Δ6,9,12
18:4 Δ6,9,12,15
20:3 Δ11,14,17
Δ8-desaturase
Δ6-elongase
Δ8-desaturase
20:3 Δ8,11,14
20:4 Δ8,11,14,17
Δ5-desaturase
20:4 Δ5,8,11,14
ARA
20:5 Δ5,8,11,14,17
EPA
Δ5-elongase
22:4 Δ7,10,13,16
22:5 Δ7,10,13,16,19
ω3-desaturase
Δ4-desaturase
22:5 Δ4,7,10,13,16
22:6 Δ4,7,10,13,16,19
DHA
ω3-desaturase
C
Sprecher-pathway
Δ7-elongase
24:5 Δ9,12,15,18,21
Δ6-desaturase
24:6 Δ6,9,12,15,18,21
β-oxidation

Fig. 2

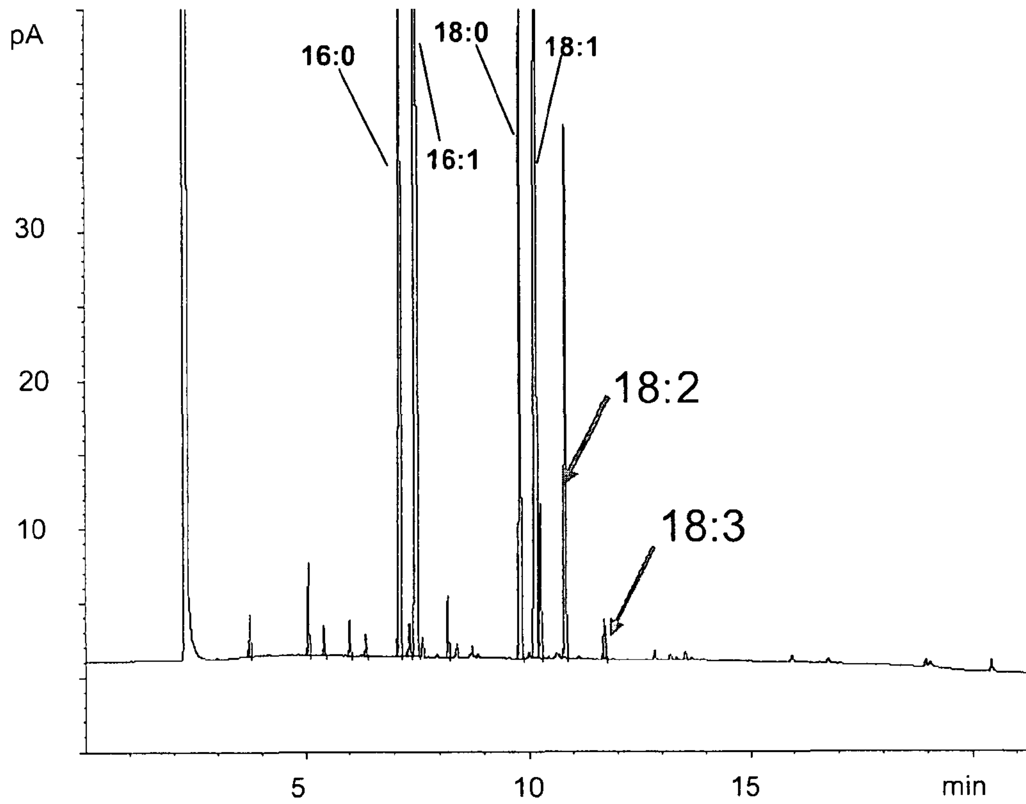
```
                   *        20         *        40         *        60         *        80
SEQ_ID_2  : ---------------MTTTRPP-----IPSRLAFDSPAGSASNTSLSSMDEQPSVRKNGKRSSGKLLDTYGNEFSIPDY :  59
SEQ_ID_4  : ---------------------------------MAVRQRTSSTTTTLYVEKPATTVIVEP-------VTQILPSPIFP :  38
SEQ_ID_6  : -MATTAMAQNPVLRRNVTATSTASSSAPSVAVSPNDSPAQSPSSTSLSSMASVEPEVKNSR---GKLIDTYGNEFSIPDY :  76
SEQ_ID_8  : MATTTARAQAPAMKRNVTTDSSPST---HANNSPFDSPAGSASNTSLSSLDESPAQSKSNR---GVLLDTYGNEFSIPDY :  74
SEQ_ID_32 : ------------------------------------------------------------------------------- :   -
SEQ_ID_34 : ---------------------------------MIASTQSKTAVTDRGTTRMRGS------------STAQEFP :  29
SEQ_ID_36 : ---------------------------------------------MAPNITSSP--------TSVLSDDTI :  19
SEQ_ID_38 : ---------------------------------------------MAPNITSTP--------TSVLSEDFA :  19

                   *       100         *       120         *       140         *       160
SEQ_ID_2  : TIKDFRDAIPKHCFERSAVRGLGYVARDLASAATFYVFHNYVTPETVPSMPSRAALWTLYTVLQGFFGTGLWILAHECGH : 139
SEQ_ID_4  : DIKSIKDAIPAHCFQPSLFTSYYYVVRDFTMVCTLVWAALTFIP--SIPDPILRGAAWMLYGFVQGLFCTGIWILGHECGH : 116
SEQ_ID_6  : TIKQIYDAIPKHCFERSAIRSLSYVARDVAVASVFYVFYNYVTPEYVPSTPSRAVLWALYTVVQGLFGTGVWVLAHECGH : 156
SEQ_ID_8  : TIKQIRDAIPKHCFERSGLRGLGYVARDIASAAVFYVFHNYVTPETVPSTPARAGLWALYTVIQGLFGTGLWVLAHECGH : 154
SEQ_ID_32 : -------------------------------------------------------------------------GHECGH :   6
SEQ_ID_34 : DIQTIRDAIPKHCFEPSTVRSLSYVARDVTMASALIWAAVRLIP--QIEDSVLRFSAWMLYGLVQGMVCTGVWILAHECGH : 107
SEQ_ID_36 : ETVTLPTIETLTDDSLPAEKYKRKLVWRNVILFVYLHMAALYGAYLMLTSCKWITAIFAFLLYQAIGLGITAG-AHRLWS :  98
SEQ_ID_38 : AAEKATSTETVEG-IAPKREYKKQLVWPNVILHFILHVGAVYGAYLMFTSAKWLTGIFAFVLYEVSGLGITAG-AHRLWS :  97

                   *       180         *       200         *       220         *       240
SEQ_ID_2  : HQAFSESKLLNDTVGLVCHSILLVPYFSWKISHGKHHKATGHMERDMVFVPKTREETYATRVGKMVHELSEWTEEAPLAT : 218
SEQ_ID_4  : HGAFSLHNKVNDIIGALLHSFLLVPYFSWKFSHHKHHKLYHNHMERDMAFVPATEPKTSRQTMLAGIGLDEWFEDTPLFQ : 195
SEQ_ID_6  : HQAFSPSKTLNDTVGLICHSALLVPYFSWKISHGKHHKATGHMTRDMVFVPKTREEYASRIGKFVHEWHEWTEETPLAT : 235
SEQ_ID_8  : HQSFSPSKVLNDTVGLFCHSALLVPYFSWKISHGKHHKATGHMERDMVFVPRTREEVYASRIGKMIHELDEWTEETPLAT : 233
SEQ_ID_32 : HQAFSPSKVLNDTVGLICHSALLVPYFSWKISHGKHHKATGNMARDMVFVPRTREEHAHRVGGTIEQLGEWMEETPLAT :  85
SEQ_ID_34 : HGAFSKHQTLNDFVGLVLHSSGLVPYFSWKFSHHKHHKFTGNMERDMVFVPAVKTEEPPKRRLASFYLDPELLDAVPLVS : 187
SEQ_ID_36 : HRSYKAKWPLRLILVIFNTLAFQNHVYEWARDHRVHHKFS---------------------------------ETDADPHN : 146
SEQ_ID_38 : HRSYKATWQMRLIL MICQTVSFQTSVHEWARNHRVHHKHS---------------------------------DTDGDPHN : 145

                   *       260         *       280         *       300         *       320
SEQ_ID_2  : LIHTFGQSLGGWPLYLITNVTGHNHHDRQIEGKGKGKKNGFFGGVNHFFPLEKLDEHLIKLSDLSVWGIVIGFVTLYW : 298
SEQ_ID_4  : TIRLIGHSLFGWQIYLFTNASGKGKGSMQREVG-----GISKWLRVSHFDPLAVRPNEAIFILLSDVGLMATVYILYQ : 270
SEQ_ID_6  : ATHMLGQSLACWLLYIFINVTGHNQHEKQKEGKGVGKKNGWFGGVNHMFPLEKIDEKLIKLSDLSVWGTGYVAYQV : 315
SEQ_ID_8  : LIHSLGQSLAGWPLYLILNVTGHNYHERQAEGKGKGKHNGPGGGVNHFFPLERLDEHLIKLSDLSVWGTLGALTLYW : 313
SEQ_ID_32 : AINLLLQSLFGWPMYLTTNVTGHNNHTKQIEGRGKGKANGWFGGVNHNFPLEAVDSEAKYIALSDLSVWGTGSAVYLY :  165
SEQ_ID_34 : LIQLLAHSLAGWQMYLLFNLSGKDSKQRNQ--------SGWLRVSHFDPLAVRPSEAWYIALSDLAVTGAALYIG :  259
SEQ_ID_36 : AKRGFFFSHVGWLLVRKHPDVK-VK--------------SKGIDMSDLDADPLIAFQHKHYLILMPIICFILPTIIPVYF :  211
SEQ_ID_38 : VNRGLFFSHAGWMMCRKHPEVK-EK--------------AKQIDLSDLDADPILMFQLKYYLILMPFMCFFLPTWIPVYF :  210

                   *       340         *       360         *       380         *       400
SEQ_ID_2  : GKNWLPNNYLWLIMLWNIGMYILMYQHTDPASFLYQADTLTLVGAHIPLHERVPAHRALTTALHIFETVRTTEMYYVY : 378
SEQ_ID_4  : SQKIVVSTTLLLGYMWHAAIMLHHHADPEYTPEGTEVVLALVLAEVPHYRALFHVIKKSEHVLF : 350
SEQ_ID_6  : GSKFLKANLLWLIVWWNIAIVQHTDPASFLYDAATLTLAFAHIEHPVSEPFLHIVKTTLAYY : 395
SEQ_ID_8  : GKNFLKANLLWLIVWWNIAIVQHTDPDSFLYDANTLTAYATIPMHEHPPMFLHIVKTEAYY : 393
SEQ_ID_32 : GSTYLWLNLLMWGMWWNAIVQHTDPTSFPYTPESLNLAGALIDLYDLRHVKTQPYY : 245
SEQ_ID_34 : STLVLWPTMFVLFWWNYAIVHETHPEHIPYEADGLTVVLAVLDLLDVPFHLIGFRAYAIF : 339
SEQ_ID_36 : WGETWSNAWLVAMFRLTETLNASWLVNSAAH-MWGSRPYDKYINPSEN---LGVSMLALGEGWHNYHHVFPWDYKTAEY : 287
SEQ_ID_38 : WGETWHNAYLAAIFRHSETLNMTSMVNSLTHNTWGNRPYDKNINPAEN---LIVSLMTLGEGWHNYHHVFPWDYKTAEL : 287

                   *       420         *       440         *       460         *       480
SEQ_ID_2  : STHDVEVKKVKRSDTKGGPNGLMNAVKTARWCQWLPSAEAELEGLGLFFYRNGLGVPPTKIEP- : 457
SEQ_ID_4  : PRKDAEALPVKLVRDSR----SLLGQLSVFGSLKYLHDPTRPLAMLWAK------------------ : 408
SEQ_ID_6  : STNDVEIKPVRERADVEDGPLGLKALKSARWCQWLPSAEAQLEGLGLFFYRNGLGVPPVVIPAP : 475
SEQ_ID_8  : STHDVEIKPVRERSDVEGGPIGLKALKSARWCQWLPSADAELEGLGLFFYHNGLGVPPQKLSAP : 473
SEQ_ID_32 : SNNDVEIQKVLSRTEAQTGWTGLFKALTSARVCQWLPSEGTTLNQGLMFFYTNGLGVPPTKMAK- : 324
SEQ_ID_34 : AKYEVAITQPVLNRHRAPG----SLLGDLLTFTKCRPLKDPEHPLAMLWLAPRLL------------- : 402
SEQ_ID_36 : GNYSTNLTTAFIDFFSRIGWAYDLKTVPMSMVKQRVQRTGDGSHDVWGWGDKDMSQEDMDEALVIAKKLK---------- : 357
SEQ_ID_38 : GVLRINMTTLFIDLCAKIGWAYDLKTVPMDMVKKRRVERTGDGTHEIWGWGDKDMTEKESEAQIIAKKD----------- : 356

                   *       500
SEQ_ID_2  : -AGTK--KAAKMEVGPESDNE-- : 475
SEQ_ID_4  : ----------------------- :   -
SEQ_ID_6  : ----GTEKKAGMIVGSDSDNDA- : 493
SEQ_ID_8  : VAKSTAGQRAKMEVGPESDNE-- : 494
SEQ_ID_32 : ----------------------- :   -
SEQ_ID_34 : ----------------------- :   -
SEQ_ID_36 : ----------------------- :   -
SEQ_ID_38 : ----------------------- :   -
```

Fig. 3

A:



B:



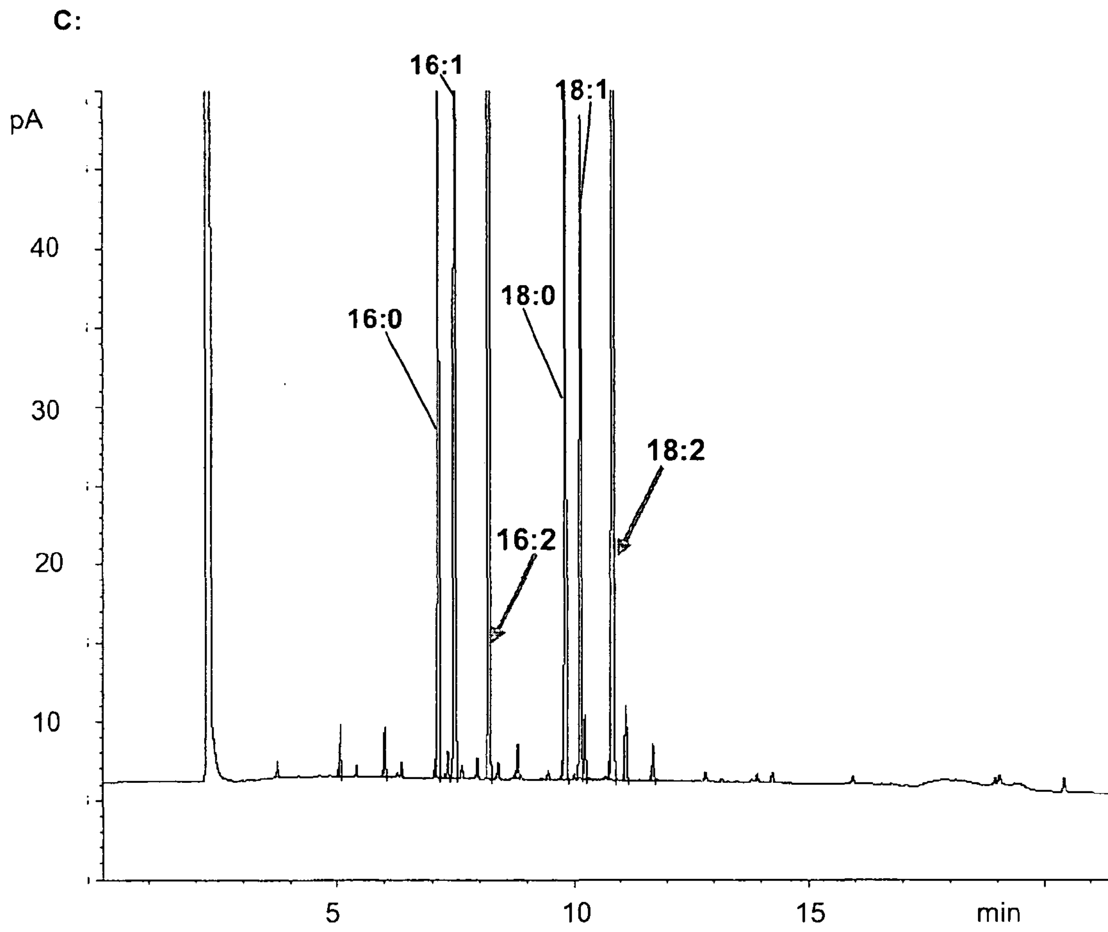
No Feeding:

16:0	16:1	18:0	18:1c9	18:1c11	18:2
18.63	40.95	7.39	19.36	1.65	7.35

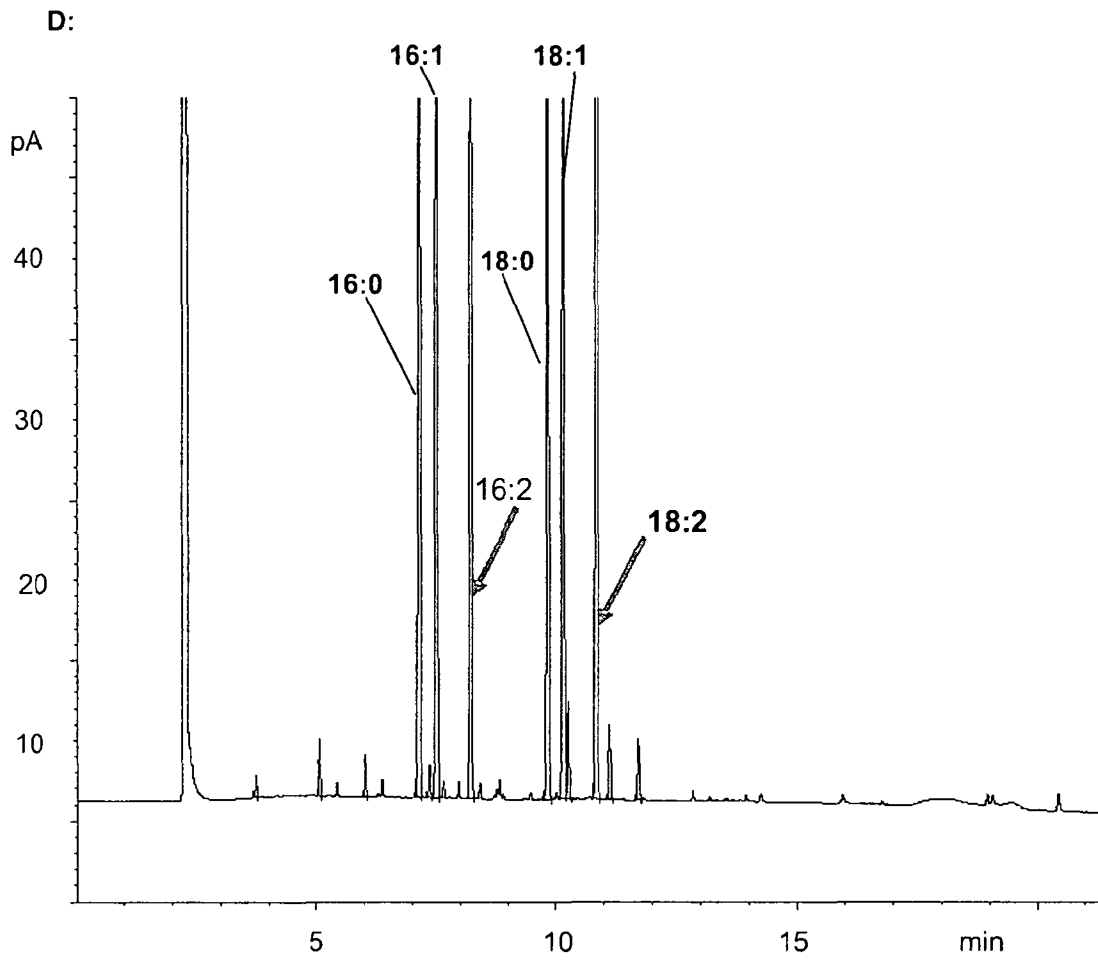
Feeding 18:2:

16:0	16:1	18:0	18:1c9	18:1c11	18:2	18:3
17.78	20.66	8.32	16.87	0.76	28.44	1.91

C:



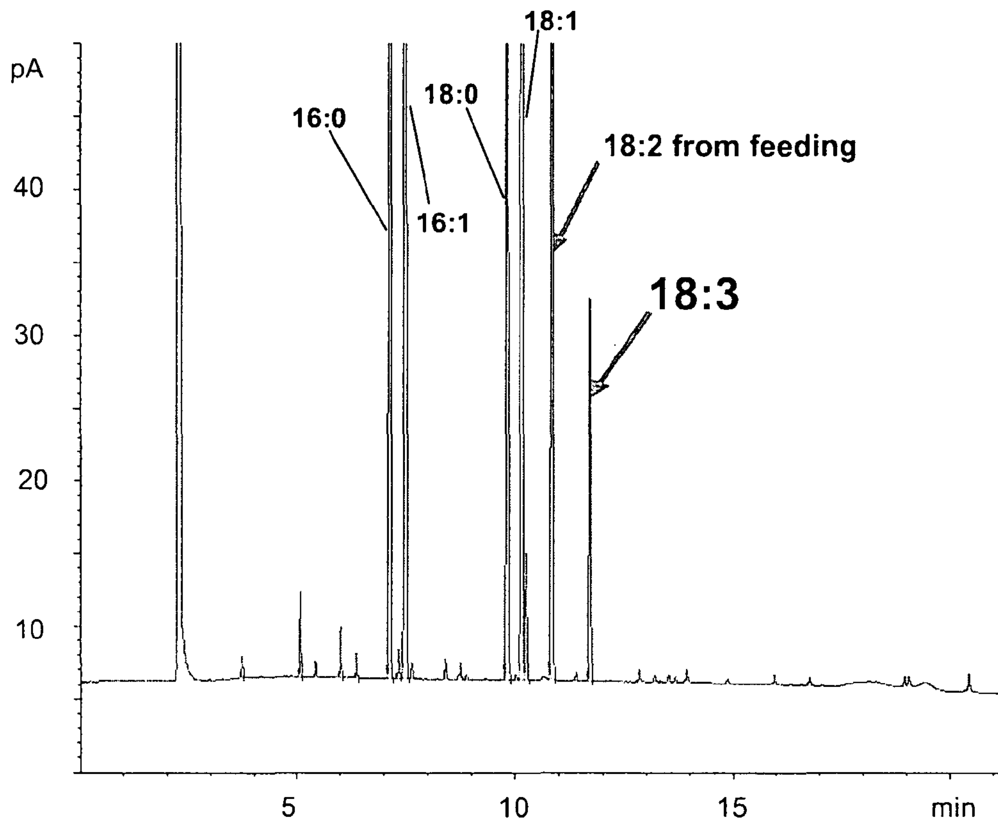
No Feeding:	16:0	16:1	16:2	18:0	18:1c9	18:1c11	18:2
	16.72	16.16	15.74	10.03	7.81	0.70	25.10



No Feeding:	16:0	16:1	16:2	18:0	18:1c9	18:1c11	18:2
	16.41	22.42	10.15	9.62	15.80	0.90	17.49

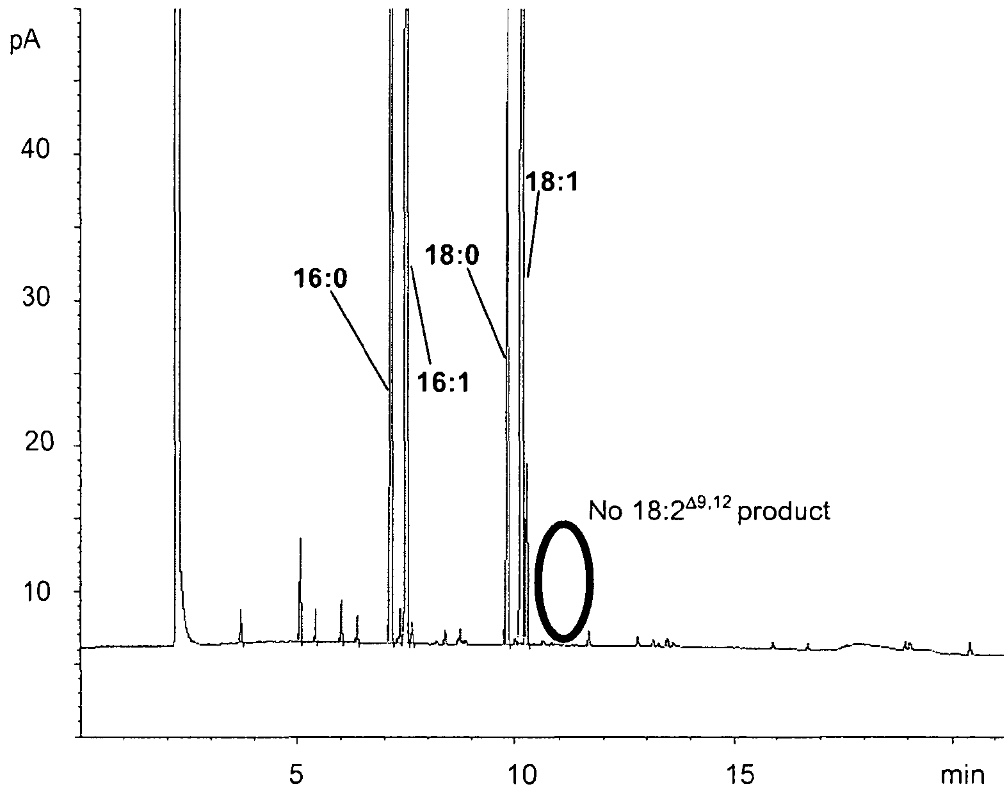
Fig. 4.

A:



Feeding 18:2:	16:0	16:1	18:0	18:1c9	18:1c11	18:2	18:3
	17.55	21.42	7.70	21.48	0.87	22.42	3.92

B:



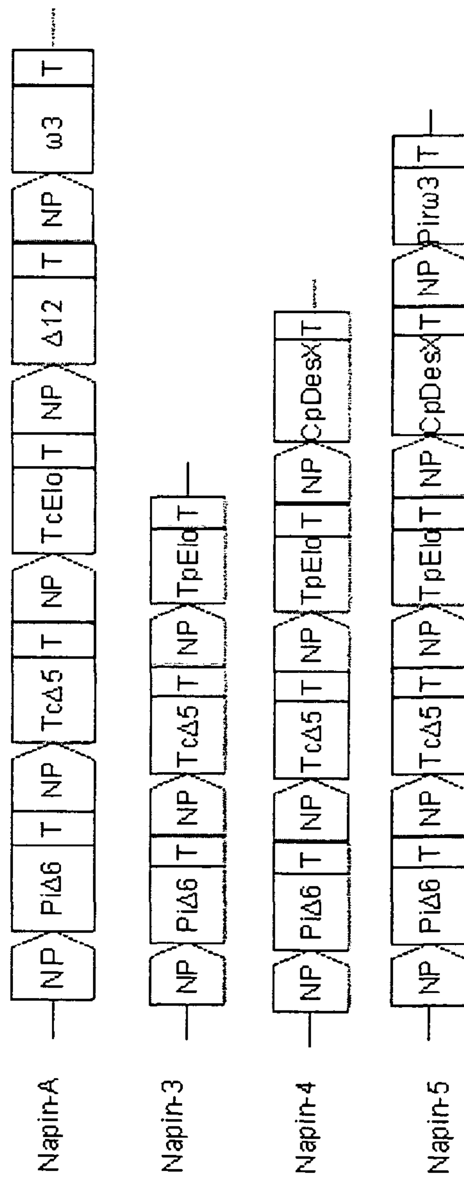


Fig. 5

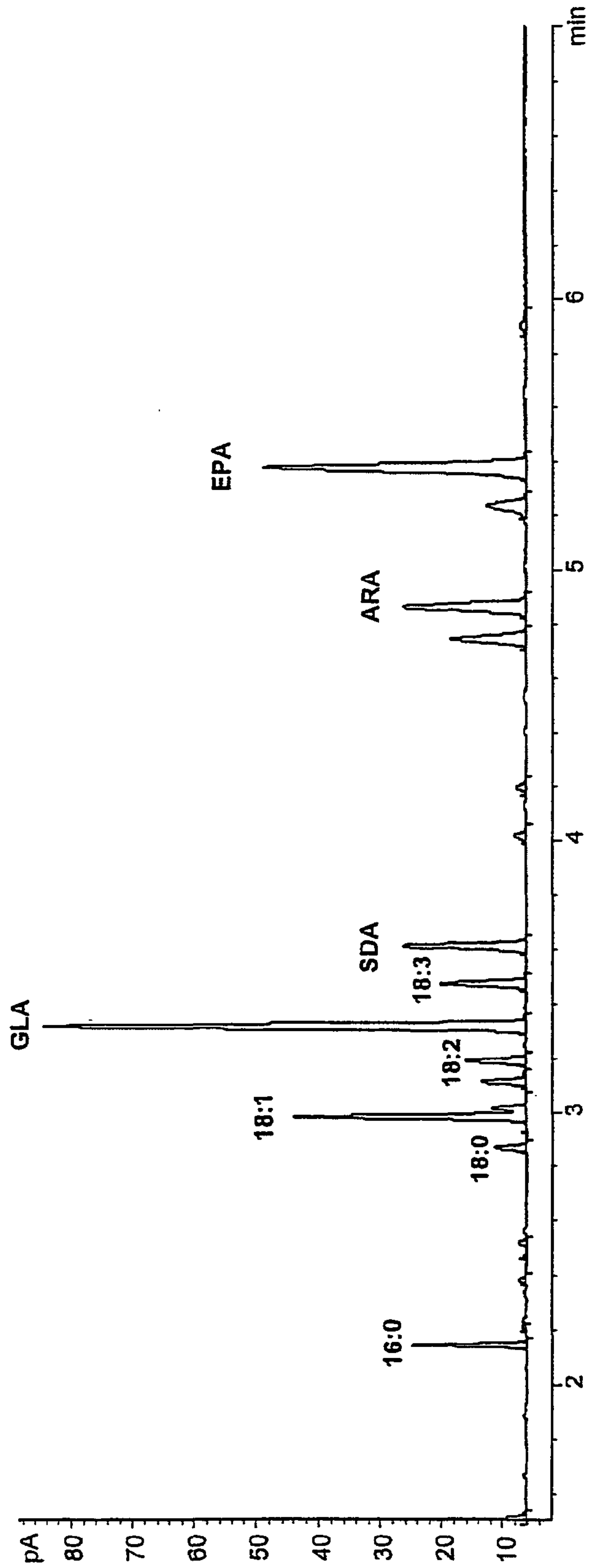


Fig. 6