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(57) Abstract: The present invention relates to a polynucleotide encoding an omega 3 (ω-3) desaturase from Pythium irregulare with specificity to long chain polyunsaturated omega 6 (ω- 6) fatty acids as well as a vector containing said polynucleotide, and a host cell containing the vector or the polynucleotide. Moreover, the present invention pertains to a polypeptide encoded by the said polynucleotide, antibodies against the polypeptide as well as a method for the manufacture of the polypeptide. Further, encompassed by the present invention are transgenic non-human organisms. Finally, the present invention relates to methods for the manufacture of compounds and oil- fatty acid- or lipid- containing compositions.

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Isolation and characterization of a novel Pythium omega 3 desaturase with specificity to all omega 6 fatty acids longer than 18 carbon chains

Description

The present invention relates to a polynucleotide encoding an omega 3 (ω-3) desaturase from Pythium irregulare with specificity to long chain polyunsaturated omega 6 (ω-6) fatty acids as well as a vector containing said polynucleotide, and a host cell containing the vector or the polynucleotide. Moreover, the present invention pertains to a polypeptide encoded by the said polynucleotide, antibodies against the polypeptide as well as a method for the manufacture of the polypeptide. Further, encompassed by the present invention are transgenic non-human organisms. Finally, the present invention relates to methods for the manufacture of compounds and oil- fatty acid- or lipid-containing compositions.

Fatty acids and triacylglycerides have a multiplicity of applications in the food industry, in animal nutrition, in cosmetics and 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 various different applications.

Polyunsaturated long-chain ω-3-fatty acids such as eicosapentaenoic acid (= EPA, $C20:5^{\Delta 5,8,11,14,17}$), ω-3 eicostetraenic acid (= ETA, $C20:4^{\Delta 8,11,14,17}$) or docosahexaenoic acid (= DHA, $C22:6^{\Delta 4,7,10,13,16,19}$) are important components of human nutrition owing to their various roles in health aspects, including the development of the child brain, the functionality of the eyes, the synthesis of hormones and other signal substances, and the prevention of cardiovascular disorders, cancer and diabetes (Poulos, A Lipids 30:1-14, 1995; Horrocks, LA and Yeo YK Pharmacol Res 40:211-225, 1999). There is, therefore, a need for the production of polyunsaturated long-chain fatty acids.

Owing to the present-day composition of human food, an addition of polyunsaturated ω-3-fatty acids, which are preferentially found in fish oils, to the food is particularly important. Thus, for example, polyunsaturated fatty acids such as DHA or EPA are added to infant formula to improve the nutritional value. The unsaturated fatty acid DHA is supposed to have a positive effect on the development and maintenance of brain functions.

In the following, polyunsaturated fatty acids are sometimes referred to as PUFA, PUFAs, LCPUFA or LCPUFAs (**p**oly **u**nsaturated **f**atty **a**cids, **PUFA**, **l**ong **c**hain **p**oly **u**nsaturated **f**atty **a**cids, **LCPUFA**).

The various fatty acids and triglycerides are mainly obtained from microorganisms such as Mortierella or Schizochytrium or from oil-producing plants such as soybeans, oilseed rape, algae such as Crypthecodinium or Phaeodactylum and others, being obtained, as a rule, in the form of their triacylglycerides (= triglycerides = triglycerols). However, they can also be obtained from animals, for example, fish. The free fatty acids are, advantageously, prepared by hydrolysis. Very long-chain polyunsaturated fatty acids such as DHA, EPA, arachidonic acid (= ARA, $C20:4^{\Delta 5,8,11,14}$), dihomo-γ-linolenic acid (=DGLA, $C20:3^{\Delta 8,11,14}$) or docosapentaenoic acid (DPA, $C22:5^{\Delta 7,10,13,16,19}$) are not synthesized in plants, for example in oil crops such as oilseed rape, soybeans, sunflowers and 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 ω-3-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 ω-3-fatty acids to the food. Also, ω-3-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. ω-6-fatty acids such as arachidonic acid tend to have an adverse effect on these disorders in connection with these rheumatic diseases on account of our usual dietary intake.

ω-3- and ω-6-fatty acids are precursors of tissue hormones, known as eicosanoids, such as the prostaglandins. The prostaglandins which are derived from dihomo-γ-linolenic acid, arachidonic acid and eicosapentaenoic acid, and of the thromoxanes and leukotrienes, which are derived from arachidonic acid and eicosapentaenoic acid. Eicosanoids (known as the PG_2 series) which are formed from the ω-6-fatty acids generally promote inflammatory reactions, while eicosanoids (known as the PG_3 series) from ω-3-fatty acids have little or no proinflammatory effect. Therefore, food having a high proportion of ω-3-fatty acid has a positive effect on human health.

Owing to their positive characteristics, there has been no lack of attempts in the past to make available genes which are involved in the synthesis of 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 describe a Δ9–desaturase. WO 93/11245 claims a Δ15-desaturase and WO 94/11516 a Δ12-desaturase. Further desaturases are described, for example, in EP A 0 550 162, WO 94/18337, WO 97/30582, WO 97/21340, WO 95/18222, EP A 0 794 250, Stukey et al., J. Biol. Chem., 265, 1990: 20144–20149, Wada et al., Nature 347, 1990: 200–203 or Huang et al., 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 al., Methods in Enzymol. 71, 1981: 12141–12147, Wang et al., 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–Desaturases are described in WO 93/06712, US 5,614,393, US 5,614,393, WO 96/21022, WO 00/21557 and WO 99/27111, and also the application for the production in transgenic organisms is described in WO 98/46763, WO 98/46764 and WO 98/46765. Here, the expression of various desaturases is also described and claimed in WO 99/64616 or WO 98/46776, as is the formation of polyunsaturated fatty acids. 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. Furthermore, mixtures of ω-3- and ω-6-fatty acids are usually obtained.

Especially suitable microorganisms for the production of PUFAs are microorganisms including microalgae such as Phaeodactylum tricornutum, Porphiridium species, Thraustochytrium species, Schizochytrium species or Crypthecodinium species, ciliates such as Stylonychia or Colpidium, fungi 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. Thus, recombinant methods are preferred wherever possible. However, only limited amounts of the desired polyunsaturated fatty acids such as ETA, DHA or EPA can be produced with the aid of the abovementioned microorganisms; where they are generally obtained as fatty acid mixtures of, for example, ETA, EPA and DHA, depending on the microorganism used.

A variety of synthetic pathways is being discussed for the synthesis of the polyunsaturated fatty acids, eicosapentaenoic acid and docosahexaenoic acid. EPA or DHA are produced in numerous marine bacteria such as Vibrio sp. or Shewanella sp. via the so-called polyketide pathway (Yu, R. et al. Lipids 35:1061-1064, 2000; Takeyama, H. et al. Microbiology 143:2725-2731, 1197)).

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 1486:219-231) 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. What is known as the Sprecher pathway 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. ω-6- or ω-3-fatty acids, which differ with regard to their metabolic and functional activities.

The starting material for the ω-6-metabolic pathway is the fatty acid linoleic acid ($18:2^{\Delta 9,12}$) while the ω-3-pathway proceeds via linolenic acid ($18:3^{\Delta 9,12,15}$). Linolenic acid is formed by the activity of an ω-3-desaturase (Tocher et al. 1998, Prog. Lipid Res. 37, 73-117; Domergue et al. 2002, Eur. J. Biochem. 269, 4105-4113).

Mammals, and thus also humans, have no corresponding desaturase activity (Δ12- and ω-3-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 ω-6-fatty acid and the two ω-3-fatty acids eicosapentaenoic acid (= EPA, $20:5^{\Delta 5,8,11,14,17}$) and docosa-hexaenoic acid (DHA, $22:6^{\Delta 4,7,10,13,17,19}$) are synthesized via the sequence of desaturase and elongase reac-

tions. The application of ω-3-fatty acids shows the therapeutic activity described above in the treatment of cardiovascular diseases (Shimikawa 2001, World Rev. Nutr. Diet. 88, 100-108), inflammations (Calder 2002, Proc. Nutr. Soc. 61, 345-358) and arthritis (Cleland and James 2000, J. Rheumatol. 27, 2305-2307).

From the angle of nutritional physiology, it is, therefore, important to achieve a shift between the ω-6-synthetic pathway and the ω-3-synthetic pathway (see Figure 1) in the synthesis of polyunsaturated fatty acids so that more ω-3-fatty acids are produced. The enzymatic activities of various ω-3-desaturases which desaturate $C_{18:2}$-, $C_{22:4}$- or $C_{22:5}$-fatty acids have been described in the literature (see Figure 1). However, none of the desaturases whose biochemistry has been described converts a broad range of substrates of the ω-6-synthetic pathway into the corresponding fatty acids of the ω-3-synthetic pathway.

There is therefore still a great demand for an ω-3-desaturase which is suitable for the production of ω-3-polyunsaturated fatty acids. All the known plant and cyanobacterial ω-3-desaturases desaturate C18-fatty acids with linoleic acid as the substrate, but cannot desaturate C20- or C22-fatty acids.

An ω-3-desaturase which can desaturate C20-polyunsaturated fatty acids is known from the fungus Saprolegnia dicilina (Pereira et al. 2003, Biochem. J. 2003 Dez, manuscript BJ20031319). However, it is disadvantageous that this ω-3-desaturase cannot desaturate C18- or C22-PUFAs, such as the important fatty acids C18:2-, C22:4- or C22:5-fatty acids of the ω-6-synthetic pathway. A further disadvantage of this enzyme is that it cannot desaturate fatty acids which are bound to phospholipids. Only the CoA-fatty acid esters are converted. Recently, other ω-3-desaturases have been described with a pivotal substrate specificity for ARA, DGLA and Docosatetraenoic acid (=DTA $^{\Delta 8,11,14,17}$ (WO2005/083053).

To make possible the fortification of food and/or of feed with polyunsaturated ω-3-fatty acids, there is still a great need for a simple, inexpensive process for the production of each of the aforementioned long chain polyunsaturated fatty acids, especially in eukaryotic systems.

The technical problem underlying the present invention, thus, could be seen as the provision of means and methods which allow the synthesis of LCPUFAs and which

allow a shift from the ω-6-synthetic pathway to the ω-3-synthetic pathway in order to manufacture polyunsaturated fatty acids and derivatives thereof. The technical problem has been solved by the embodiments characterized below and in the accompanying claims.

Accordingly, the present invention relates to a polynucleotide comprising a nucleic acid sequences selected from the group consisting of:

(a) a nucleic acid sequence as shown in SEQ ID NO: 1 or 23;

(b) a nucleic acid sequence encoding a polypeptide having an amino acid sequence as shown in SEQ ID NO: 2 or 24;

(c) a nucleic acid sequence which is at least 70% identical to the nucleic acid sequence of (a) or (b), wherein said nucleic acid sequence encodes a polypeptide having ω-3 desaturase activity;

(d) a nucleic acid sequence being a fragment of any one of (a) to (c), wherein said fragment encodes a polypeptide having ω-3 desaturase activity; and

(e) a nucleic acid sequence encoding a polypeptide having ω-3 desaturase activity, wherein saif polypeptide comprises a polypeptide pattern as shown in a sequence selected from the group consisting of SEQ ID NO: 15, 16, 17, 18, 19, 20, 21, 22, 37, 38, 39, 40, 41, 42, 43, 44 and 45.

The term “polynucleotide” as used in accordance with the present invention relates to a polynucleotide comprising a nucleic acid sequence which encodes a polypeptide having ω-3 desaturase activity, i.e. being capable of converting a ω-6 PUFA into its corresponding ω-3 PUFA. More preferably, the polypeptide encoded by the polynucleotide of the present invention shall be capable of introducing a double bond on the ω-3-position into a ω-6 PUFA. The ω-6 PUFA is, preferably, an LCPUFA, more preferably, a C20- or C22-PUFA. C20- and C22-PUFAs are also referred to as LCPUFAs herein below. Most preferably, the polynucleotide of the present invention encodes a polypeptide which is capable of converting ω-6 DPA into DHA. Suitable assays for measuring the activities mentioned before are described in the accompanying Examples or in WO2005/083053. A polynucleotide encoding a polypeptide having the aforementioned biological activity has been obtained in accordance with the present invention from Py-

thium irregulare. Thus, the polynucleotide, preferably, comprises the nucleic acid sequence shown in SEQ ID NO: 1 or 23 encoding the polypeptide having an amino acid sequence as shown in SEQ ID NO: 2 or 24, respectively. The two polypeptides shall represent isoforms of the ω3-desaturase of the present invention. It is to be understood that a polypeptide having an amino acid sequence as shown in SEQ ID NO: 2 or 24 may be also encoded due to the degenerated genetic code by other polynucleotides as well.

Moreover, the term “polynucleotide” as used in accordance with the present invention further encompasses variants of the aforementioned specific polynucleotides. Said variants may represent orthologs, paralogs or other homologs of the polynucleotide of the present invention. Homolgous polynucleotides are, preferably, polynucleotides comprise sequences as shown in any one of SEQ ID NO: 6, 7, 9, 11, 13, 30, 33 or 35 or those which encode polypeptides comprising amino acid sequences as shown in any one of SEQ ID NOs: 8, 10, 12, 14, 31, 34 or 36.

The polynucleotide variants, preferably, also 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 or 23 or in any one of SEQ ID NOs: 6, 7, 9, 11, 13, 30, 33 or 35 by at least one nucleotide substitution, addition and/or deletion whereby the variant nucleic acid sequence shall still encode a polypeptide having ω-3 desaturase activity as specified above. Variants also encompass polynucleotides comprising a nucleic acid sequence which is capable of hybridizing to the aforementioned specific nucleic acid sequences, preferably, under stringent hybridization conditions. These stringent conditions are known to the skilled worker and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y. (1989), 6.3.1-6.3.6. A preferred example for stringent hybridization conditions are hybridization conditions in 6 × sodium chloride/sodium citrate (= SSC) at approximately 45°C, followed by one or more wash steps in 0.2 × SSC, 0.1% SDS at 50 to 65°C. The skilled worker knows that these hybridization conditions differ depending on the type of nucleic acid and, for example when organic solvents are present, with regard to the temperature and concentration of the buffer. For example, under “standard hybridization conditions” the temperature differs depending on the type of nucleic acid between 42°C and 58°C in aqueous buffer with a concentration of 0.1 to 5 × SSC (pH 7.2). If organic solvent is present in the abovementioned buffer, for example 50% formamide, the temperature under standard conditions is approximately 42°C. The hybridization conditions for DNA:DNA hybrids are, preferably, 0.1 × SSC and 20°C to 45°C, preferably between 30°C and 45°C. The hybridization conditions for DNA:RNA hybrids are, preferably, 0.1 × SSC and 30°C to 55°C, preferably between 45°C and 55°C. The abovementioned hybridization temperatures are determined for example for a nucleic acid with approxi-

mately 100 bp (= base pairs) in length and a G + C content of 50% in the absence of formamide. The skilled worker knows how to determine the hybridization conditions required by referring to textbooks such as the textbook mentioned above, or the following textbooks: Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989; Hames and Higgins (Ed.) 1985, "Nucleic Acids Hybridization: A Practical Approach", IRL Press at Oxford University Press, Oxford; Brown (Ed.) 1991, "Essential Molecular Biology: A Practical Approach", IRL Press at Oxford University Press, Oxford. Alternatively, polynucleotide variants are obtainable by PCR-based techniques such as mixed oligonucleotide primer- based amplification of DNA, i.e. using degenerated primers against conserved domains of the polypeptides of the present invention. Conserved domains of the polypeptide of the present invention may be identified by a sequence comparison of the nucleic acid sequence of the polynucleotide or the amino acid sequence of the polypeptide of the present invention with other ω-3 desaturase sequences (see, e.g., Figure 3). Oligonucleotides suitable as PCR primers as well as suitable PCR conditions are described in the accompanying Examples. As a template, DNA or cDNA from bacteria, fungi, plants or animals may be used. Further, variants include polynucleotides comprising nucleic acid sequences which are at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the nucleic acid sequences shown in SEQ ID NO: 1 or 23 retaining ω-3 desaturase activity. Moreover, also encompassed are polynucleotides which comprise nucleic acid sequences encoding amino acid sequences which are at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequences shown in SEQ ID NO: 2 or 24 or an amino acid sequence as shown in any one of SEQ ID NOs: 8, 10, 12, 14, 31, 34 or 36 wherein the polypeptide comprising the amino acid sequence retains ω-3 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: 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 53711 (1991)], are to be used. The sequence identity values recited above in percent (%) are to be determined, preferably, using the program 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.

A polynucleotide comprising a fragment of any of the aforementioned nucleic acid sequences is also encompassed as a polynucleotide of the present invention. The fragment shall encode a polypeptide which still has ω-3 desaturase activity as specified above. Accordingly, the polypeptide may comprise or consist of the domains of the polypeptide of the present invention conferring the said biological activity. A fragment as meant herein, preferably, comprises at least 50, at least 100, at least 250 or at least 500 consecutive nucleotides of any one of the aforementioned nucleic acid sequences or encodes an amino acid sequence comprising at least 20, at least 30, at least 50, at least 80, at least 100 or at least 150 consecutive amino acids of any one of the aforementioned amino acid sequences.

The variant polynucleotides or fragments referred to above, preferably, encode polypeptides retaining 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 ω-3 desaturase activity exhibitited by the polypeptide shown in SEQ ID NO: 2 or 24. The activity may be tested as described in the accompanying Examples.

Further varaiant polynucleotides encompassed by the present invention comprise sequence motifs as shown in any one of SEQ ID NOs: 15, 16, 17, 18, 19, 20, 21, 22, 37, 38, 39, 40, 41, 42, 43, 44 or 45. The depicted sequences show amino acid sequence patterns (also referred to as polypeptide patterns) which are required for a polynucleotide in order to encode a polypeptide having ω-3 desaturase activity as specified above and, in particular, for those polypeptides being capable of converting ω-6 DPA into DHA. In principle, a polypeptide pattern as referred to in accordance with the present invention comprises, preferably, less than 100 or less than 50, more preferably, at least 10 up to 30 or at least 15 up to 20 amino acid in length. Moreover, it is to be understood that a variant polynucleotide comprised by the present invention, preferably, comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen or all of the aforementioned specific sequence motifs. Accordingly, the pattern as shown in SEQ ID NO: 15 may be combined with the pattern shown in SEQ ID NO: 16, the pattern as shown in SEQ ID NO: 16 may be combined with the pattern shown in SEQ ID NO: 17, the pattern as shown in SEQ ID NO: 17 may be combined with the pattern shown in SEQ ID NO: 18, the pattern as shown in SEQ ID NO: 18 may be combined with the pattern shown in SEQ ID NO: 19, the pattern as shown in SEQ ID NO: 19 may be combined with the pattern shown in SEQ ID NO: 20. Likewise, the pattern as shown in SEQ ID NO: 37 may be combined with the pattern shown in SEQ ID NO: 38, the pattern as shown in SEQ ID NO: 38 may be combined with the pattern shown in SEQ ID NO: 49, the pattern as shown in SEQ ID NO: 22 may be combined with the pattern shown in

SEQ ID NO: 37 or the pattern as shown in SEQ ID NO: 20 may be combined with the pattern shown in SEQ ID NO: 37 and the pattern as shown in SEQ ID NO: 44. In principle, all permutations for the combination of pairs or groups of up to seventeen patterns based on the aforementioned sequence pattern are envisaged by the present invention.

The polynucleotides of the present invention either essentially consist of the aforementioned nucleic acid sequences or comprise the aforementioned nucleic acid sequences. Thus, they may contain further nucleic acid sequences as well. Preferably, the polynucleotide of the present invention may comprise further untranslated sequence at the 3' and at the 5' terminus of the coding gene region: at least 500, preferably 200, more preferably 100 nucleotides of the sequence upstream of the 5' terminus of the coding region and at least 100, preferably 50, more preferably 20 nucleotides of the sequence downstream of the 3' terminus of the coding gene region. Furthermore, the polynucleotides of the present invention may encode fusion proteins wherein one partner of the fusion protein is a polypeptide being encoded by a nucleic acid sequence recited above. Such fusion proteins may comprise as additional part other enzymes of the fatty acid or 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 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, polynucleotides may be obtained by in vitro and in vivo mutagenesis approaches using the above mentioned mentioned specific polynucleotides as a basis. Moreover, polynucleotids 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 Ceratodon, or higher plants such as the Primulaceae such as Aleuritia, Calendula stellata, Osteospermum spinescens or Osteospermum hyoseroides, microorganisms such as fungi, such as Aspergillus, Thraustochytrium, Phytophthora, Entomophthora, Mucor or Mortierella, bacteria such as Shewanella, yeasts or animals. Preferred animals are nematodes such as Caenorhabditis, insects or vertebrates. Among the vertebrates, the polynucleotides may, preferably, be derived from Euteleostomi, Actinopterygii; Neopterygii; Teleostei; Euteleo-

stei, Protacanthopterygii, Salmoniformes; Salmonidae or Oncorhynchus, more preferably, from the order of the Salmoniformes, most preferably, the family of the Salmonidae, such as the genus Salmo, for example from the genera and species Oncorhynchus mykiss, Trutta trutta or Salmo trutta fario. Moreover, the polynucleotides may be obtained from the diatoms such as the genera Thallasiosira or Crypthecodinium.

The polynucleotide of the present invention shall be provided, preferably, either as an isolated polynucleotide (i.e. isolated from its natural context such as a gene locus) or in genetically modified form. An isolated polynucleotide can, for example, comprise less than approximately 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in the genomic DNA of the cell from which the nucleic acid is derived. The polynucleotide, preferably, is double or single stranded DNA including cDNA or RNA. The term encompasses single as well as double stranded polynucleotides. Moreover, comprised are also chemically modified polynucleotides including naturally occurring modified polynucleotides such as glycosylated or methylated polynucleotides or artificial modified ones such as biotinylated polynucleotides.

Advantageously, it has been found in the studies underlying the present invention that the polypeptides being encoded by the polynucleotides of the present invention have ω-3 desaturse activity and, in particular, are capable of converting ω-6 LCPUFA substrates, such as C20- and C22-PUFAs, into the corresponding ω-3 PUFAs. As shown in Table 1 in the accompanying Examples, the conversion of ARA into EPA is catalyzed with the highest efficiency (more than 40%). However, the conversion of DGLA into ETA is also catalyzed. Remarkably, the enzymes encoded by the polynucleotides of the present invention are even capable of catalyzing the conversion of DPA into DHA. The polynucleotides of the present invention are, in principle, useful for the synthesis of LCPUFAs and compositions containing such compounds. Specifically, thanks to the present invention, LCPUFAs and, in particular, even DHA can be recombinantly manufactured using transgenic organisms, such as micro-organisms, plants and animals.

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

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 polynucleotides of the present invention, preferably, further comprises selectable markers for propagation and/or selection in a host. The vector may be incorporated into a host cell by various techniques well known in the art. If introduced into a host cell, the vector may reside in the cytoplasm or may be incorporated into the genome. In the latter case, it is to be understood that the vector may further comprise nucleic acid sequences which allow for homologous recombination or heterologous insertion. Vectors can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. The terms “transformation” and “transfection”, conjugation and transduction, as used in the present context, are intended to comprise a multiplicity of prior-art processes for introducing foreign nucleic acid (for example DNA) into a host cell, including calcium phosphate, rubidium chloride or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, natural competence, carbon-based clusters, chemically mediated transfer, electroporation or particle bombardment. Suitable methods for the transformation or transfection of host cells, including plant cells, can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) and other laboratory manuals, such as Methods in Molecular Biology, 1995, Vol. 44, Agrobacterium protocols, Ed.: Gartland and Davey, Humana Press, Totowa, New Jersey. Alternatively, a plasmid vector may be introduced by heat shock or electroporation techniques. Should the vector be a virus, it may be packaged in vitro using an appropriate packaging cell line prior to application to host cells. 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 microbial systems. Such vectors ensure efficient cloning in bacteria and, preferably, yeasts or fungi and make possible the stable transformation of plants. Those which must be mentioned are, in particular, various binary and co-integrated vector systems which are suitable for the T-DNA-mediated transformation. Such vector systems are, as a rule, characterized in that they contain at least the vir genes, which are required for the Agrobacterium-mediated transformation, and the sequences which delimit the T-DNA (T-DNA border). These vector systems, preferably, also comprise further cis-

regulatory regions such as promoters and terminators and/or selection markers with which suitable transformed host cells or organisms can be identified. While co-integrated vector systems have vir genes and T-DNA sequences arranged on the same vector, binary systems are based on at least two vectors, one of which bears vir genes, but no T-DNA, while a second one bears T-DNA, but no vir gene. As a consequence, the last-mentioned vectors are relatively small, easy to manipulate and can be replicated both in E. coli and in Agrobacterium. These binary vectors include vectors from the pBIB-HYG, pPZP, pBecks, pGreen series. Preferably used in accordance with the invention are Bin19, pBI101, pBinAR, pGPTV and pCAMBIA. An overview of binary vectors and their use can be found in Hellens et al, Trends in Plant Science (2000) 5, 446–451. Furthermore, by using appropriate cloning vectors, the polynucleotides can be introduced into host cells or organisms such as plants or animals and, thus, be used in the transformation of plants, such as those which are published, and cited, in: Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida), chapter 6/7, pp. 71-119 (1993); F.F. White, Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, 15-38; B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press (1993), 128-143; Potrykus, 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 polynucleotide 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 polynucleotide, preferably, into a translatable mRNA. 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 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 *AOX1* 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 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 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 polynucleotides or vector of the invention into targeted cell population. Methods 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).

Suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (Invitrogene) or pSPORT1 (GIBCO BRL). Further examples of typical fusion expression vectors are pGEX (Pharmacia Biotech Inc; Smith, D.B., and Johnson, K.S. (1988) Gene 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ), where glutathione S-transferase (GST), maltose E-binding protein and protein A, respectively, are fused with the recombinant target protein. Examples of suitable inducible nonfusion E. coli expression vectors are, inter alia, pTrc (Amann et al. (1988) Gene 69:301-315) and pET 11d (Studier et al., Gene Expression Technology: 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 from the pET 11d vector is based on the transcription of a T7-gn10-lac fusion promoter, which is mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is provided by the host strains BL21 (DE3) or HMS174 (DE3) from a resident λ-prophage which harbors a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter. The skilled worker is familiar with other vectors which are suitable in prokaryotic organisms; these vectors are, for example, in E. coli, pLG338, pACYC184, the pBR series such as pBR322, the pUC series such as pUC18 or pUC19, the M113mp series, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III113-B1, λgt11 or pBdCI, in Streptomyces pIJ101, pIJ364, pIJ702 or pIJ361, in Bacillus pUB110, pC194 or pBD214, in Corynebacterium pSA77 or pAJ667. Examples of vectors for expression in the yeast S. cerevisiae comprise pYeDesaturasec1 (Baldari et al.

(1987) Embo J. 6:229-234), pMFa (Kurjan and Herskowitz (1982) Cell 30:933-943), pJRY88 (Schultz et al. (1987) Gene 54:113-123) and pYES2 (Invitrogen Corporation, San Diego, CA). Vectors and processes for the construction of vectors which are suitable for use in other fungi, such as the filamentous fungi, comprise those which are described in detail in: van den Hondel, C.A.M.J.J., & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of fungi, J.F. Peberdy et al., Ed., pp. 1-28, Cambridge University Press: Cambridge, or in: More Gene Manipulations in Fungi (J.W. Bennett & L.L. Lasure, Ed., pp. 396-428: Academic Press: San Diego). Further suitable yeast vectors are, for example, pAG-1, YEp6, YEp13 or pEMBLYe23. As an alternative, the polynucleotides of the present invention can be also expressed in insect cells using baculovirus expression vectors. Baculovirus vectors which are available for the expression of proteins in cultured insect cells (for example Sf9 cells) comprise the pAc series (Smith et al. (1983) Mol. Cell Biol. 3:2156-2165) and the pVL series (Lucklow and Summers (1989) Virology 170:31-39).

The following promoters and expression control sequences may be, preferably, used in an expression vector according to the present invention. The cos, tac, trp, tet, trp-tet, lpp, lac, lpp-lac, laclq, T7, T5, T3, gal, trc, ara, SP6, λ-PR or λ-PL promoters are, preferably, used in Gram-negative bacteria. For Gram-positive bacteria, promoters amy and SPO2 may be used. From yeast or fungal promoters ADC1, MFα, 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 promoter. 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 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-0 249 676. Particularly preferred are promoters which enable the expression in tissues which are involved in the biosynthesis of fatty acids. Also particularly preferred are seed-specific promoters such as the USP promoter in accordance with the practice, but also other promoters such as the LeB4, DC3, phaseolin or napin promoters. Further especially 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

(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 and other promoters which are suitable and which are described in WO 99/16890. In principle, it is possible to use all natural promoters together with their regulatory sequences, such as those mentioned above, for the novel process. Likewise, it is possible and advantageous to use synthetic promoters, either additionally or alone, especially when they mediate a seed-specific expression, such as, for example, as described in WO 99/16890.

The polynucleotides 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 Spermatophytes, 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. 20:1195-1197; and Bevan, M.W. (1984) “Binary Agrobacterium vectors for plant transformation”, Nucl. Acids Res. 12:8711-8721; Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, p. 15-38. A plant expression cassette, preferably, comprises regulatory sequences which are capable of controlling the gene expression in plant cells and which are functionally linked so that each sequence can fulfill its function, such as transcriptional termination, for example polyadenylation signals. Preferred polyadenylation signals are those which are derived from Agrobacterium tumefaciens T-DNA, such as the gene 3 of the Ti plasmid pTiACH5, which is known as octopine synthase (Gielen et al., 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 mosaic virus leader sequence, which increases the protein/RNA ratio (Gallie et al., 1987, Nucl. Acids Research 15:8693-8711). As described above, plant gene expression must be functionally linked to a suitable promoter which performs the expression of the gene in a timely, cell-specific or tissue-specific manner. Promoters which can be used are

constitutive promoters (Benfey et al., EMBO J. 8 (1989) 2195-2202) such as those 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 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 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 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 promoters which are especially preferred are those which bring about the expression of genes in tissues and organs in which fatty acid, lipid and oil biosynthesis takes place, in seed cells such as the cells of endosperm and of the developing embryo. Suitable promoters are the napin gene promoter from oilseed rape (US 5,608,152), the USP promoter from Vicia faba (Baeumlein 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 com-

partment 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.

In a preferred embodiment of the vector of the present invention, the said vector comprises at least one polynucleotide encoding a further enzyme being involved in the biosynthesis of fatty acids or lipids. A further enzyme referred to in accordance with the present invention is, preferably, selected from the group consisting of acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyltransferase(s), acyl-CoA:lysophospholipid acyltransferase(s), fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenase(s), lipoxygenase(s), triacylglycerol lipase(s), allenoxide synthase(s), hydroperoxide lyase(s) or fatty acid elongase(s), acyl-CoA:lysophospholipid acyltransferase, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ8-desaturase, Δ9-desaturase, Δ12-desaturase, Δ5-elongase, Δ6-elongase and Δ9-elongase. Most preferably, the vector comprises at least one polynucleotide encoding an enzyme selected from the group consisting of Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ8-desaturase, Δ9-desaturase, Δ12-desaturase, Δ5-elongase, Δ6-elongase and Δ9-elongase in addition to at least one polynucleotide encoding an enzyme selected from the group consisting of acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyltransferase(s), acyl-CoA:lysophospholipid acyltransferase(s), fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenase(s), lipoxygenase(s), triacylglycerol lipase(s), allenoxide synthase(s), hydroperoxide lyase(s) or fatty acid elongase(s), and acyl-CoA:lysophospholipid acyltransferase. The at least one polynucleotide encoding said further enzyme may be obtained from any bacteria, fungi,

animal or plant and, preferably, from those specifically recited in this description. Preferably, each polynucleotide encoding a further enzyme as recited above is also linked to its own expression control sequence wherein said expression control sequences may or may not be identical. The vector of the present invention, thus, preferably, comprises at least two (i.e. the expression cassette for the polynucleotide of the present invention and the polynucleotide for the at least one further enzyme) up to a plurality of expression cassettes consisting of the polynucleotides and expression control sequences operatively linked expression control sequences thereto.

The invention also pertains to a host cell comprising the polynucleotide or the vector of the present invention.

Host cells are primary cells or cell lines derived from multicellular organisms such as plants or animals. Furthermore, host cells encompass prokaryotic or eukaryotic single cell organisms (also referred to as micro-organisms). Primary cells or cell lines to be used as host cells in accordance with the present invention may be derived from the multicellular organisms referred to below. Host cells which can be exploited are furthermore mentioned in: Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). Specific expression strains which can be used, for example those with a lower protease activity, are described in: Gottesman, S., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 119-128. These include plant cells and certain tissues, organs and parts of plants in all their phenotypic forms such as anthers, fibers, root hairs, stalks, embryos, calli, cotelydons, petioles, harvested material, plant tissue, reproductive tissue and cell cultures which is derived from the actual transgenic plant and/or can be used for bringing about the transgenic plant. Preferably, the host cells may be obtained from plants. More preferably, oil crops are envisaged which comprise large amounts of lipid compounds, such as oilseed rape, evening primrose, hemp, thistle, peanut, canola, linseed, soybean, safflower, sunflower, borage, or plants such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, Tagetes, Solanaceae plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa, bushy plants (coffee, cacao, tea), Salix species, trees (oil palm, coconut) and perennial grasses and fodder crops. Especially preferred plants according to the invention are oil crops such as soybean, peanut, oilseed rape, canola, linseed, hemp, evening primrose, sunflower, safflower, trees (oil palm, coconut). Suitable methods for ob-

taining host cells from the multicellular organisms referred to below as well as conditions for culturing these cells are well known in the art.

The micro-organisms are, preferably, bacteria or fungi including yeasts. Preferred fungi to be used in accordance with the present invention are selected from the group of the families Chaetomiaceae, Choanephoraceae, Cryptococcaceae, Cunninghamellaceae, Demetiaceae, Moniliaceae, Mortierellaceae, Mucoraceae, Pythiaceae, Sacharomycetaceae, Saprolegniaceae, Schizosacharomycetaceae, Sodariaceae or Tuberculariaceae. Further preferred micro-organisms are selected from the group: Choanephoraceae such as the genera Blakeslea, Choanephora, for example the genera and species *Blakeslea trispora, Choanephora cucurbitarum, Choanephora infundibulifera* var. *cucurbitarum,* Mortierellaceae, such as the genus Mortierella, for example the genera and species *Mortierella isabellina, Mortierella polycephala*, *Mortierella ramanniana*, *Mortierella vinacea, Mortierella zonata,* Pythiaceae such as the genera Phytium, Phytophthora for example the genera and species *Pythium debaryanum, Pythium intermedium, Pythium irregulare, Pythium megalacanthum, Pythium paroecandrum, Pythium sylvaticum, Pythium ultimum, Phytophthora cactorum, Phytophthora cinnamomi, Phytophthora citricola, Phytophthora citrophthora, Phytophthora cryptogea, Phytophthora drechsleri, Phytophthora erythroseptica, Phytophthora lateralis, Phytophthora megasperma, Phytophthora nicotianae, Phytophthora nicotianae var. parasitica, Phytophthora palmivora, Phytophthora parasitica, Phytophthora syringae*, Saccharomycetaceae such as the genera Hansenula, Pichia, Saccharomyces, Saccharomycodes, Yarrowia for example the genera and species *Hansenula anomala, Hansenula californica, Hansenula canadensis, Hansenula capsulata, Hansenula ciferrii, Hansenula glucozyma, Hansenula henricii, Hansenula holstii, Hansenula minuta, Hansenula nonfermentans, Hansenula philodendri, Hansenula polymorpha, Hansenula saturnus, Hansenula subpelliculosa, Hansenula wickerhamii, Hansenula wingei, Pichia alcoholophila, Pichia angusta, Pichia anomala, Pichia bispora, Pichia burtonii, Pichia canadensis, Pichia capsulata, Pichia carsonii, Pichia cellobiosa, Pichia ciferrii, Pichia farinosa, Pichia fermentans, Pichia finlandica, Pichia glucozyma, Pichia guilliermondii, Pichia haplophila, Pichia henricii, Pichia holstii, Pichia jadinii, Pichia lindnerii, Pichia membranaefaciens, Pichia methanolica, Pichia minuta var. minuta, Pichia minuta var. nonfermentans, Pichia norvegensis, Pichia ohmeri, Pichia pastoris, Pichia philodendri, Pichia pini, Pichia polymorpha, Pichia quercuum, Pichia rhodanensis, Pichia sargentensis, Pichia stipitis, Pichia strasburgensis, Pichia subpelliculosa, Pichia toletana, Pichia trehalophila, Pichia vini, Pichia xylosa, Saccharomyces aceti, Saccharomyces bailii, Saccharomyces bayanus, Saccharomyces bisporus, Saccharomyces capensis,*

Saccharomyces carlsbergensis, Saccharomyces cerevisiae, Saccharomyces cerevisiae var. ellipsoideus, Saccharomyces chevalieri, Saccharomyces delbrueckii, Saccharomyces diastaticus, Saccharomyces drosophilarum, Saccharomyces elegans, Saccharomyces ellipsoideus, Saccharomyces fermentati, Saccharomyces florentinus, Saccharomyces fragilis, Saccharomyces heterogenicus, Saccharomyces hienipiensis, Saccharomyces inusitatus, Saccharomyces italicus, Saccharomyces kluyveri,Saccharomyces krusei, Saccharomyces lactis, Saccharomyces marxianus, Saccharomyces microellipsoides, Saccharomyces montanus, Saccharomyces norbensis, Saccharomyces oleaceus, Saccharomyces paradoxus, Saccharomyces pastorianus, Saccharomyces pretoriensis, Saccharomyces rosei, Saccharomyces rouxii, Saccharomyces uvarum, Saccharomycodes ludwigii, Yarrowia lipolytica, Schizosacharomycetaceae such as the genera Schizosaccharomyces e.g. the species *Schizosaccharomyces japonicus var. japonicus, Schizosaccharomyces japonicus var. versatilis, Schizosaccharomyces malidevorans, Schizosaccharomyces octosporus, Schizosaccharomyces pombe var. malidevorans, Schizosaccharomyces pombe var. pombe,* Thraustochytriaceae such as the genera Althornia, Aplanochytrium, Japonochytrium, Schizochytrium, Thraustochytrium e.g. the species *Schizochytrium aggregatum, Schizochytrium limacinum, Schizochytrium mangrovei, Schizochytrium minutum, Schizochytrium octosporum, Thraustochytrium aggregatum, Thraustochytrium amoeboideum, Thraustochytrium antacticum, Thraustochytrium arudimentale, Thraustochytrium aureum, Thraustochytrium benthicola, Thraustochytrium globosum, Thraustochytrium indicum, Thraustochytrium kerguelense, Thraustochytrium kinnei, Thraustochytrium motivum, Thraustochytrium multirudimentale, Thraustochytrium pachydermum, Thraustochytrium proliferum, Thraustochytrium roseum, Thraustochytrium rossii, Thraustochytrium striatum* or *Thraustochytrium visurgense*. Further preferred micro-organisms are bacteria selected from the group of the families Bacillaceae, Enterobacteriacae or Rhizobiaceae. Examples of such micro-organisms may be selected from the group: Bacillaceae such as the genera Bacillus for example the genera and species *Bacillus acidocaldarius, Bacillus acidoterrestris, Bacillus alcalophilus, Bacillus amyloliquefaciens, Bacillus amylolyticus, Bacillus brevis, Bacillus cereus, Bacillus circulans, Bacillus coagulans, Bacillus sphaericus subsp. fusiformis, Bacillus galactophilus, Bacillus globisporus, Bacillus globisporus subsp. marinus, Bacillus halophilus, Bacillus lentimorbus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus polymyxa, Bacillus psychrosaccharolyticus, Bacillus pumilus, Bacillus sphaericus, Bacillus subtilis subsp. spizizenii, Bacillus subtilis subsp. subtilis* or *Bacillus thuringiensis;* Enterobacteriacae such as the genera Citrobacter, Edwardsiella, Enterobacter, Erwinia, Escherichia, Klebsiella, Salmonella or Serratia for example the genera and species

Citrobacter amalonaticus, Citrobacter diversus, Citrobacter freundii, Citrobacter genomospecies, Citrobacter gillenii, Citrobacter intermedium, Citrobacter koseri, Citrobacter murliniae, Citrobacter sp., Edwardsiella hoshinae, Edwardsiella ictaluri, Edwardsiella tarda, Erwinia alni, Erwinia amylovora, Erwinia ananatis, Erwinia aphidicola, Erwinia billingiae, Erwinia cacticida, Erwinia cancerogena, Erwinia carnegieana, Erwinia carotovora subsp. *atroseptica, Erwinia carotovora* subsp. *betavasculorum, Erwinia carotovora* subsp. *odorifera, Erwinia carotovora* subsp. *wasabiae, Erwinia chrysanthemi, Erwinia cypripedii, Erwinia dissolvens, Erwinia herbicola, Erwinia mallotivora, Erwinia milletiae, Erwinia nigrifluens, Erwinia nimipressuralis, Erwinia persicina, Erwinia psidii, Erwinia pyrifoliae, Erwinia quercina, Erwinia rhapontici, Erwinia rubrifaciens, Erwinia salicis, Erwinia stewartii, Erwinia tracheiphila, Erwinia uredovora, Escherichia adecarboxylata, Escherichia anindolica, Escherichia aurescens, Escherichia blattae, Escherichia coli, Escherichia coli var. communior, Escherichia coli-mutabile, Escherichia fergusonii, Escherichia hermannii, Escherichia sp., Escherichia vulneris, Klebsiella aerogenes, Klebsiella edwardsii subsp. atlantae, Klebsiella ornithinolytica, Klebsiella oxytoca, Klebsiella planticola, Klebsiella pneumoniae, Klebsiella pneumoniae subsp. pneumoniae, Klebsiella sp., Klebsiella terrigena, Klebsiella trevisanii, Salmonella abony, Salmonella arizonae, Salmonella bongori, Salmonella choleraesuis subsp. arizonae, Salmonella choleraesuis subsp. bongori, Salmonella choleraesuis subsp. cholereasuis, Salmonella choleraesuis subsp. diarizonae, Salmonella choleraesuis subsp. houtenae, Salmonella choleraesuis subsp. indica, Salmonella choleraesuis subsp. salamae, Salmonella daressalaam, Salmonella enterica subsp. houtenae, Salmonella enterica subsp. salamae, Salmonella enteritidis, Salmonella gallinarum, Salmonella heidelberg, Salmonella panama, Salmonella senftenberg, Salmonella typhimurium, Serratia entomophila, Serratia ficaria, Serratia fonticola, Serratia grimesii, Serratia liquefaciens, Serratia marcescens, Serratia marcescens subsp. marcescens, Serratia marinorubra, Serratia odorifera, Serratia plymouthensis, Serratia plymuthica, Serratia proteamaculans, Serratia proteamaculans subsp. quinovora, Serratia quinivorans* or *Serratia rubidaea;* Rhizobiaceae such as the genera Agrobacterium, Carbophilus, Chelatobacter, Ensifer, Rhizobium, Sinorhizobium for example the genera and species *Agrobacterium atlanticum, Agrobacterium ferrugineum, Agrobacterium gelatinovorum, Agrobacterium larrymoorei, Agrobacterium meteori, Agrobacterium radiobacter, Agrobacterium rhizogenes, Agrobacterium rubi, Agrobacterium stellulatum, Agrobacterium tumefaciens, Agrobacterium vitis, Carbophilus carboxidus, Chelatobacter heintzii, Ensifer adhaerens, Ensifer arboris, Ensifer fredii, Ensifer kostiensis, Ensifer kummerowiae, Ensifer medicae, Ensifer meliloti, Ensifer saheli, Ensifer terangae, Ensifer xinjiangensis, Rhizobium ciceri Rhizobium etli*, *Rhizobium fredii, Rhizobium galegae, Rhizobium gallicum, Rhizobium*

giardinii, Rhizobium hainanense, Rhizobium huakuii, Rhizobium huautlense, Rhizobium indigoferae, Rhizobium japonicum, Rhizobium leguminosarum, Rhizobium loessense, Rhizobium loti, Rhizobium lupini, Rhizobium mediterraneum, Rhizobium meliloti, Rhizobium mongolense, Rhizobium phaseoli, Rhizobium radiobacter, Rhizobium rhizogenes, Rhizobium rubi, Rhizobium sullae, Rhizobium tianshanense, Rhizobium trifolii, Rhizobium tropici, Rhizobium undicola, Rhizobium vitis, Sinorhizobium adhaerens, Sinorhizobium arboris, Sinorhizobium fredii, Sinorhizobium kostiense, Sinorhizobium kummerowiae, Sinorhizobium medicae, Sinorhizobium meliloti, Sinorhizobium morelense, Sinorhizobium saheli or *Sinorhizobium xinjiangense.*

How to culture the aforementioned micro-organisms is well known to the person skilled in the art.

In a preferred embodiment of the host cell of the present invention, the said host cell additionally comprises at least one further enzyme being involved in the biosynthesis of fatty acids or lipids, preferably, selected from the group consisting of: acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyltransferase(s), acyl-CoA:lysophospholipid acyltransferase(s), fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenase(s), lipoxygenase(s), triacylglycerol lipase(s), allenoxide synthase(s), hydroperoxide lyase(s) or fatty acid elongase(s), acyl-CoA:lysophospholipid acyltransferase, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ8-desaturase, Δ9-desaturase, Δ12-desaturase, Δ5-elongase, Δ6-elongase and Δ9-elongase. More preferably, the host cell comprises at least one further enzyme selected from the group consisting of Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ8-desaturase, Δ9-desaturase, Δ12-desaturase, Δ5-elongase, Δ6-elongase and Δ9-elongase in addition to at least one further enzyme selected from the group consisting of acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyltransferase(s), acyl-CoA:lysophospholipid acyltransferase(s), fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenase(s), lipoxygenase(s), triacylglycerol lipase(s), allenoxide synthase(s), hydroperoxide lyase(s) or fatty acid elongase(s), and acyl-CoA:lysophospholipid acyltransferase. The enzyme may be endogenously expressed in the host cell or may be exogenously supplied, e.g., by introducing one or more expression vector(s) comprising the polynucleotides encoding the aforementioned further enzymes.

The present invention also includes a method for the manufacture of a polypeptide having ω-3 desaturase activity comprising:

(a) expressing the polynucleotide of the present invention in a host cell as specified above; and
(b) obtaining the polypeptide encoded by said polynucleotide from the host cell.

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.

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

The term “polypeptide” as used herein encompasses essentially purified polypeptides or polypeptide preparations comprising other proteins in addition. Further, the term also relates to the fusion proteins or polypeptide fragments being at least partially encoded by the polynucleotide of the present invention referred to above. Moreover, it includes chemically modified polypeptides. Such modifications may be artificial modifications or naturally occurring modifications such as phosphorylation, glycosylation, myristylation and the like. The terms “polypeptide”, “peptide” or “protein” are used interchangeable throughout this specification. As referred to above, the polypeptide of the present invention shall exhibit ω-3 desaturase activity and, thus, can be used for the manufacture of LCPUFAs, in particular C20- or C22- LCPUFAS, either in a host cell or in a transgenic animal or plant as described elsewhere in this specification. Surprisingly, the ω-3 desaturase activity of the polypeptide of the present invention even includes the ability to convert ω-6 DPA into DHA.

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 methods using a purified polypeptide according to the invention or a suitable fragment derived therefrom as an antigen. A fragment which is suitable as an antigen may be

identified by antigenicity determining algorithms well known in the art. Such fragments may be obtained either from the polypeptide of the invention by proteolytic digestion or may be a synthetic peptide. Preferably, the antibody of the present invention is a monoclonal antibody, a polyclonal antibody, a single chain antibody, a 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 (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 Spring Harbor, 1988. Monoclonal antibodies can be prepared by the techniques originally described in Köhler and Milstein, Nature 256 (1975), 495, and Galfré, 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.

The present invention relates to a transgenic non-human organism comprising the polynucleotide, the vector or the host cell of the present invention.

The term “non-human transgenic organism”, preferably, relates to a plant, an animal or a multicellular micro-organism. The polynucleotide or vector may be present in the cytoplasm of the organism or may be incorporated into the genome either heterologous or by homologous recombination. Host cells, in particular those obtained from plants or animals, may be introduced into a developing embryo in order to obtain mosaic or chimeric organisms, i.e. non-human transgenic organisms comprising the host cells of the present invention. Preferably, the non-human transgenic organism expresses the polynucleotide of the present invention in order to produce the polypeptide in an amount resulting in a detectable ω-3 desaturase activity. Suitable transgenic organisms are, preferably, all those organisms which are capable of synthesizing fatty acids, spe-

cifically unsaturated fatty acids, or which are suitable for the expression of recombinant genes.

Preferred animals to be used for making non-human transgenic organisms according to the present invention include mammals, reptiles, birds, fishes, insects and worms. Preferred mammals are rodents such as mice, rabbits or rats or farming animals such as cows, pigs, sheep or goats. Preferred fishes are derived from the classes of the Euteleostomi, Actinopterygii; Neopterygii; Teleostei; Euteleostei, Protacanthopterygii, Salmoniformes; Salmonidae or Oncorhynchus and, more preferably, from the order of the Salmoniformes, in particular, the family of the Salmonidae, such as the genus Salmo, for example from the genera and species Oncorhynchus mykiss, Trutta trutta or Salmo trutta fario. Preferred insects are flies such as the fruitfly Drosophila melanogaster and preferred worms may be from the family of Caenorhabditae.

A method for the production of a transgenic non-human animal comprises introduction of the polynucleotide or vector of the present invention into a germ cell, an embryonic cell, embryonic stem (ES) cell or an egg or a cell derived therefrom. Production of transgenic embryos and screening of those can be performed, e.g., as described by A. L. Joyner Ed., Gene Targeting, A Practical Approach (1993), Oxford University Press. Genomic DNA of embryonic tissues may be analyzed for the presence of the polynucleotide or vector of the present invention by hybridization-based or PCR-based techniques. A general method for making transgenic non-human animals is described in the art, see for example WO 94/24274. For making transgenic non-human organisms (which include homologously targeted non-human animals), ES cells are preferred. Details on making such transgenic non-human organisms are described in Robertson, E. J. (1987) in Teratocarcinomas and Embryonic Stem Cells: A Practical Approach. E. J. Robertson, ed. (Oxford: IRL Press), p. 71-112. Methods for producing transgenic insects, such as Drosophila melanogaster, are also known in the art, see for example US 4,670,388, Brand & Perrimon, Development (1993) 118: 401-415; and Phelps & Brand, Methods (April 1998) 14: 367-379. Transgenic nematodes such as C. elegans can be generated as described in Mello, 1991, Embo J 10, 3959-70 or Plasterk, 1995 Methods Cell Biol 48, 59-80.

Preferred plants to be used for making non-human transgenic organisms according to the present invention are plants which are capable of synthesizing fatty acids, such as all dicotyledonous or monocotyledonous plants, algae or mosses. Advantageous plants are selected from the group of the plant families Adelotheciaceae, Anacardiaceae, As-

teraceae, 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 Tagetes. Examples which may be mentioned are the following plants selected from the group consisting of: Adelotheciaceae such as the genera Physcomitrella, such as the genus and species *Physcomitrella patens,* Anacardiaceae such as the genera Pistacia, Mangifera, Anacardium, for example the genus and species *Pistacia vera* [pistachio], *Mangifer indica* [mango] or *Anacardium occidentale* [cashew], Asteraceae, such as the genera Calendula, Carthamus, Centaurea, Cichorium, Cynara, Helianthus, Lactuca, Locusta, Tagetes, Valeriana, for example the genus and species Calendula officinalis [common marigold], Carthamus tinctorius [safflower], *Centaurea cyanus* [cornflower], *Cichorium intybus* [chicory], Cynara scolymus [artichoke], *Helianthus annus* [sunflower], *Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola L. ssp. sativa, Lactuca scariola L. var. integrata, Lactuca scariola L. var. integrifolia, Lactuca sativa subsp. romana*, *Locusta communis, Valeriana locusta* [salad vegetables], *Tagetes lucida, Tagetes erecta* or *Tagetes tenuifolia* [african or french marigold], Apiaceae, such as the genus Daucus, for example the genus and species *Daucus carota* [carrot], Betulaceae, such as the genus Corylus, for example the genera and species *Corylus avellana* or *Corylus colurna* [hazelnut], Boraginaceae, such as the genus Borago, for example the genus and species *Borago officinalis* [borage], Brassicaceae, such as the genera Brassica, Melanosinapis, Sinapis, Arabadopsis, for example the genera and species *Brassica napus*, *Brassica rapa* ssp. [oilseed rape], *Sinapis arvensis Brassica juncea, Brassica juncea var. juncea, Brassica juncea var. crispifolia, Brassica juncea var. foliosa, Brassica nigra, Brassica sinapioides, Melanosinapis communis* [mustard], *Brassica oleracea* [fodder beet] or Arabidopsis thaliana, Bromeliaceae, such as the genera Anana, Bromelia (pineapple), for example the genera and species *Anana comosus, Ananas ananas* or *Bromelia comosa* [pineapple], Caricaceae, such as the genus Carica, such as the genus and species *Carica papaya* [pawpaw], Cannabaceae, such as the genus Cannabis, such as the genus and species *Cannabis sativa* [hemp], Convolvulaceae, such as the genera Ipomea, Convolvulus, for example the genera and species *Ipomoea batatus, Ipomoea pandurata, Convolvulus batatas, Convolvulus tiliaceus, Ipomoea fastigiata, Ipomoea tiliacea, Ipomoea triloba* or *Convolvulus panduratus* [sweet potato, batate], Chenopodiaceae, such as the genus Beta, such as the genera and species *Beta vulgaris, Beta vulgaris var. altissima*, *Beta vulgaris var.Vulgaris, Beta maritima, Beta vulgaris var. perennis, Beta vulgaris var. conditiva* or *Beta vulgaris*

var. esculenta [sugarbeet], Crypthecodiniaceae, such as the genus Crypthecodinium, for example the genus and species *Cryptecodinium cohnii,* Cucurbitaceae, such as the genus Cucurbita, for example the genera and species *Cucurbita maxima, Cucurbita mixta, Cucurbita pepo* or *Cucurbita moschata* [pumpkin/squash], Cymbellaceae such as the genera Amphora, Cymbella, Okedenia, Phaeodactylum, Reimeria, for example the genus and species *Phaeodactylum tricornutum,* Ditrichaceae such as the genera Ditrichaceae, Astomiopsis, Ceratodon, Chrysoblastella, Ditrichum, Distichium, Eccremidium, Lophidion, Philibertiella, Pleuridium, Saelania, Trichodon, Skottsbergia, for example the genera and species *Ceratodon antarcticus, Ceratodon columbiae, Ceratodon heterophyllus, Ceratodon purpureus, Ceratodon purpureus, Ceratodon purpureus ssp. convolutus, Ceratodon, purpureus spp. stenocarpus*, *Ceratodon purpureus var. rotundifolius, Ceratodon ratodon, Ceratodon stenocarpus, Chrysoblastella chilensis, Ditrichum ambiguum, Ditrichum brevisetum, Ditrichum crispatissimum, Ditrichum difficile, Ditrichum falcifolium, Ditrichum flexicaule, Ditrichum giganteum, Ditrichum heteromallum, Ditrichum lineare, Ditrichum lineare, Ditrichum montanum, Ditrichum montanum, Ditrichum pallidum, Ditrichum punctulatum, Ditrichum pusillum, Ditrichum pusillum var. tortile, Ditrichum rhynchostegium, Ditrichum schimperi, Ditrichum tortile, Distichium capillaceum, Distichium hagenii, Distichium inclinatum, Distichium macounii, Eccremidium floridanum, Eccremidium whiteleggei, Lophidion strictus, Pleuridium acuminatum, Pleuridium alternifolium, Pleuridium holdridgei, Pleuridium mexicanum, Pleuridium ravenelii, Pleuridium subulatum, Saelania glaucescens, Trichodon borealis, Trichodon cylindricus* or *Trichodon cylindricus var. oblongus,* Elaeagnaceae such as the genus Elaeagnus, for example the genus and species *Olea europaea* [olive], Ericaceae such as the genus Kalmia, for example the genera and species *Kalmia latifolia, Kalmia angustifolia, Kalmia microphylla, Kalmia polifolia*, *Kalmia occidentalis, Cistus chamaerhodendros* or *Kalmia lucida* [mountain laurel], Euphorbiaceae such as the genera Manihot, Janipha, Jatropha, Ricinus, for example the genera and species *Manihot utilissima, Janipha manihot, Jatropha manihot, Manihot aipil, Manihot dulcis, Manihot manihot, Manihot melanobasis, Manihot esculenta* [manihot] or *Ricinus communis* [castor-oil plant], Fabaceae such as the genera Pisum, Albizia, Cathormion, Feuillea, Inga, Pithecolobium, Acacia, Mimosa, Medicajo, Glycine, Dolichos, Phaseolus, Soja, for example the genera and species *Pisum sativum, Pisum arvense, Pisum humile* [pea], *Albizia berteriana, Albizia julibrissin, Albizia lebbeck, Acacia berteriana, Acacia littoralis, Albizia berteriana, Albizzia berteriana, Cathormion berteriana, Feuillea berteriana, Inga fragrans, Pithecellobium berterianum, Pithecellobium fragrans, Pithecolobium berterianum, Pseudalbizzia berteriana, Acacia julibrissin, Acacia nemu, Albizia nemu, Feuilleea julibrissin, Mimosa julibrissin, Mimosa speciosa, Sericanrda*

julibrissin, Acacia lebbeck, Acacia macrophylla, Albizia lebbek, Feuilleea lebbeck, Mimosa lebbeck, Mimosa speciosa [silk tree], *Medicago sativa, Medicago falcata, Medicago varia* [alfalfa], *Glycine max Dolichos soja, Glycine gracilis, Glycine hispida, Phaseolus max, Soja hispida* or *Soja max* [soybean], Funariaceae such as the genera Aphanorrhegma, Entosthodon, Funaria, Physcomitrella, Physcomitrium, for example the genera and species *Aphanorrhegma serratum, Entosthodon attenuatus, Entosthodon bolanderi, Entosthodon bonplandii, Entosthodon californicus, Entosthodon drummondii, Entosthodon jamesonii, Entosthodon leibergii, Entosthodon neoscoticus, Entosthodon rubrisetus, Entosthodon spathulifolius, Entosthodon tucsoni, Funaria americana, Funaria bolanderi, Funaria calcarea, Funaria californica, Funaria calvescens, Funaria convoluta, Funaria flavicans, Funaria groutiana, Funaria hygrometrica, Funaria hygrometrica var. arctica, Funaria hygrometrica var. calvescens, Funaria hygrometrica var. convoluta, Funaria hygrometrica var. muralis, Funaria hygrometrica var. utahensis, Funaria microstoma, Funaria microstoma var. obtusifolia, Funaria muhlenbergii, Funaria orcuttii, Funaria plano-convexa, Funaria polaris, Funaria ravenelii, Funaria rubriseta, Funaria serrata, Funaria sonorae, Funaria sublimbatus, Funaria tucsoni, Physcomitrella californica, Physcomitrella patens, Physcomitrella readeri, Physcomitrium australe, Physcomitrium californicum, Physcomitrium collenchymatum, Physcomitrium coloradense, Physcomitrium cupuliferum, Physcomitrium drummondii, Physcomitrium eurystomum, Physcomitrium flexifolium, Physcomitrium hookeri, Physcomitrium hookeri var. serratum, Physcomitrium immersum, Physcomitrium kellermanii, Physcomitrium megalocarpum, Physcomitrium pyriforme, Physcomitrium pyriforme var. serratum, Physcomitrium rufipes, Physcomitrium sandbergii, Physcomitrium subsphaericum, Physcomitrium washingtoniense,* Geraniaceae, such as the genera Pelargonium, Cocos, Oleum, for example the genera and species *Cocos nucifera, Pelargonium grossularioides* or *Oleum cocois* [coconut], Gramineae, such as the genus Saccharum, for example the genus and species Saccharum officinarum, Juglandaceae, such as the genera Juglans, Wallia, for example the genera and species *Juglans regia, Juglans ailanthifolia, Juglans sieboldiana, Juglans cinerea, Wallia cinerea, Juglans bixbyi, Juglans californica, Juglans hindsii, Juglans intermedia, Juglans jamaicensis, Juglans major, Juglans microcarpa, Juglans nigra* or *Wallia nigra* [walnut], Lauraceae, such as the genera Persea, Laurus, for example the genera and species *Laurus nobilis* [bay], *Persea americana, Persea gratissima* or *Persea persea* [avocado], Leguminosae, such as the genus Arachis, for example the genus and species *Arachis hypogaea* [peanut], Linaceae, such as the genera Linum, Adenolinum, for example the genera and species *Linum usitatissimum, Linum humile, Linum austriacum, Linum bienne, Linum angustifolium, Linum catharticum, Linum flavum, Linum grandiflorum, Adenolinum grandiflo-*

rum, Linum lewisii, Linum narbonense, Linum perenne, Linum perenne var. lewisii, Linum pratense or *Linum trigynum* [linseed], Lythrarieae, such as the genus Punica, for example the genus and species *Punica granatum* [pomegranate], Malvaceae, such as the genus Gossypium, for example the genera and species *Gossypium hirsutum, Gossypium arboreum, Gossypium barbadense, Gossypium herbaceum* or *Gossypium thurberi* [cotton], Marchantiaceae, such as the genus Marchantia, for example the genera and species *Marchantia berteroana, Marchantia foliacea, Marchantia macropora*, Musaceae, such as the genus Musa, for example the genera and species *Musa nana, Musa acuminata, Musa paradisiaca, Musa* spp. [banana], Onagraceae, such as the genera Camissonia, Oenothera, for example the genera and species *Oenothera biennis* or *Camissonia brevipes* [evening primrose], Palmae, such as the genus Elacis, for example the genus and species *Elaeis guineensis* [oil palm], Papaveraceae, such as the genus Papaver, for example the genera and species *Papaver orientale, Papaver rhoeas, Papaver dubium* [poppy], Pedaliaceae, such as the genus Sesamum, for example the genus and species *Sesamum indicum* [sesame], Piperaceae, such as the genera Piper, Artanthe, Peperomia, Steffensia, for example the genera and species *Piper aduncum, Piper amalago, Piper angustifolium, Piper auritum, Piper betel, Piper cubeba, Piper longum, Piper nigrum, Piper retrofractum, Artanthe adunca, Artanthe elongata, Peperomia elongata, Piper elongatum, Steffensia elongata* [cayenne pepper], Poaceae, such as the genera Hordeum, Secale, Avena, Sorghum, Andropogon, Holcus, Panicum, Oryza, Zea (maize), Triticum, for example the genera and species *Hordeum vulgare, Hordeum jubatum, Hordeum murinum*, *Hordeum secalinum, Hordeum distichon, Hordeum aegiceras, Hordeum hexastichon, Hordeum hexastichum, Hordeum irregulare, Hordeum sativum, Hordeum secalinum* [barley], *Secale cereale* [rye], Avena sativa, *Avena fatua, Avena byzantina, Avena fatua var. sativa, Avena hybrida* [oats], *Sorghum bicolor, Sorghum halepense, Sorghum saccharatum, Sorghum vulgare, Andropogon drummondii, Holcus bicolor, Holcus sorghum, Sorghum aethiopicum, Sorghum arundinaceum, Sorghum caffrorum, Sorghum cernuum, Sorghum dochna, Sorghum drummondii, Sorghum durra, Sorghum guineense, Sorghum lanceolatum, Sorghum nervosum, Sorghum saccharatum, Sorghum subglabrescens, Sorghum verticilliflorum, Sorghum vulgare, Holcus halepensis, Sorghum miliaceum*, *Panicum militaceum [millet], Oryza sativa, Oryza latifolia* [rice], *Zea mays* [maize], *Triticum aestivum, Triticum durum, Triticum turgidum, Triticum hybernum, Triticum macha, Triticum sativum* or *Triticum vulgare* [wheat], Porphyridiaceae, such as the genera Chroothece, Flintiella, Petrovanella, Porphyridium, Rhodella, Rhodosorus, Vanhoeffenia, for example the genus and species *Porphyridium cruentum,* Proteaceae, such as the genus Macadamia, for example the genus and species *Macadamia intergrifolia* [macadamia],

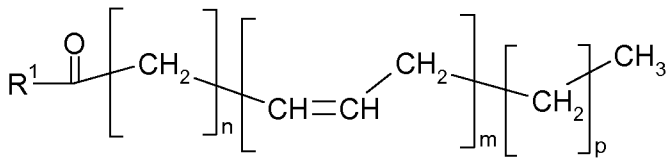
Prasinophyceae such as the genera Nephroselmis, Prasinococcus, Scherffelia, Tetraselmis, Mantoniella, Ostreococcus, for example the genera and species *Nephroselmis olivacea, Prasinococcus capsulatus, Scherffelia dubia*, *Tetraselmis chui, Tetraselmis suecica,* Mantoniella squamata, Ostreococcus tauri, Rubiaceae such as the genus Cofea, for example the genera and species Cofea spp., *Coffea arabica, Coffea canephora* or *Coffea liberica* [coffee], Scrophulariaceae such as the genus Verbascum, for example the genera and species *Verbascum blattaria, Verbascum chaixii, Verbascum densiflorum, Verbascum lagurus, Verbascum longifolium, Verbascum lychnitis, Verbascum nigrum, Verbascum olympicum, Verbascum phlomoides, Verbascum phoenicum, Verbascum pulverulentum* or *Verbascum thapsus* [mullein], Solanaceae such as the genera Capsicum, Nicotiana, Solanum, Lycopersicon, for example the genera and species *Capsicum annuum, Capsicum annuum var. glabriusculum, Capsicum frutescens* [pepper], *Capsicum annuum* [paprika], *Nicotiana tabacum, Nicotiana alata, Nicotiana attenuata, Nicotiana glauca, Nicotiana langsdorffii, Nicotiana obtusifolia, Nicotiana quadrivalvis, Nicotiana repanda, Nicotiana rustica, Nicotiana sylvestris* [tobacco], *Solanum tuberosum* [potato], *Solanum melongena* [eggplant], *Lycopersicon esculentum, Lycopersicon lycopersicum, Lycopersicon pyriforme, Solanum integrifolium* or *Solanum lycopersicum* [tomato], Sterculiaceae, such as the genus Theobroma, for example the genus and species *Theobroma cacao* [cacao] or Theaceae, such as the genus Camellia, for example the genus and species *Camellia sinensis* [tea]. In particular preferred plants to be used as transgenic plants in accordance with the present invention are oil fruit crops which comprise large amounts of lipid compounds, such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, sesame, Calendula, Punica, evening primrose, mullein, thistle, wild roses, hazelnut, almond, macadamia, avocado, bay, pumpkin/squash, linseed, soybean, pistachios, borage, trees (oil palm, coconut, walnut) or crops such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, Tagetes, Solanaceae plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa or bushy plants (coffee, cacao, tea), Salix species, and perennial grasses and fodder crops. Preferred plants according to the invention are oil crop plants such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, Calendula, Punica, evening primrose, pumpkin/squash, linseed, soybean, borage, trees (oil palm, coconut). Especially preferred are plants which are high in C18:2- and/or C18:3-fatty acids, such as sunflower, safflower, tobacco, mullein, sesame, cotton, pumpkin/squash, poppy, evening primrose, walnut, linseed, hemp, thistle or safflower. Very especially preferred plants are plants such as safflower, sunflower, poppy, evening primrose, walnut, linseed, or hemp.

Preferred mosses are Physcomitrella or Ceratodon. Preferred algae are Isochrysis, Mantoniella, Ostreococcus or Crypthecodinium, and algae/diatoms such as Phaeodactylum or Thraustochytrium. More preferably, said algae or mosses are selected from the group consisting of: Shewanella, Physcomitrella, Thraustochytrium, Fusarium, Phytophthora, Ceratodon, Isochrysis, Aleurita, Muscarioides, Mortierella, Phaeodactylum, Cryphthecodinium, specifically from the genera and species 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 tricornutum 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), chapter 6/7, pp.71-119 (1993); F.F. White, Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, 15-38; B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press (1993), 128-143; Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991), 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.

Preferably, a multicellular micro-organism as used herein refers to protists or diatoms. More preferably, it is selected from the group of the families Dinophyceae, Turaniellidae or Oxytrichidae, such as the genera and species: *Crypthecodinium cohnii, Phaeodactylum tricornutum, Stylonychia mytilus, Stylonychia pustulata, Stylonychia putrina, Stylonychia notophora, Stylonychia sp.,* Colpidium campylum or Colpidium sp.

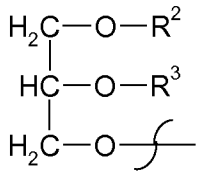
The present invention further encompasses a method for the manufacture of a compound having a structure as shown in the general formula I



(I)

wherein the variables and substituents in formula I are

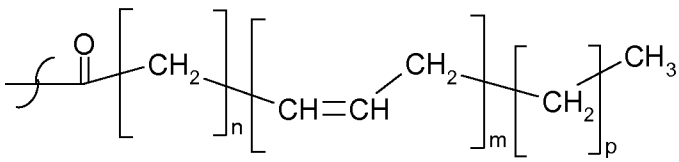
R^1 = hydroxyl, coenzyme A (thioester), lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidylglycerol, lysophosphatidylserine, lysophosphatidylinositol, sphingo base or a radical of the formula II



(II),

R^2 = hydrogen, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidylglycerol, lysophosphatidylserine, lysophosphatidylinositol or saturated or unsaturated C_2-C_{24}-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:



(Ia)

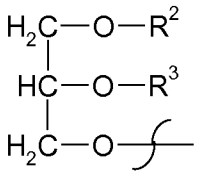
n = 2, 3, 4, 5, 6, 7 or 9, m = 2, 3, 4, 5 or 6 and p = 0 or 3;

and

wherein said method comprises cultivating (i) the host cell of any of claims 3 to 5, (ii) the transgenic non-human organism of claim 12 or 13 or (iii) a host cell or a transgenic non-human organism comprising a polynucleotide comprising a nucleic acid sequence as shown in any one of SEQ ID NOs: 6, 7, 9, 11, 13, 30, 33 or 35 or which encodes a polypeptide having an amino acid sequence as shown in any one of SEQ ID NOs: 8, 10, 12, 14, 31, 34 or 36 under conditions which allow biosynthesis of the said com-

pound, preferably, with a content of at least 1% by weight of these compounds based on the total lipid content of the host cell or the transgenic non-human organism.

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



(II).

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

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 C_{10}-C_{22}-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 C_{10}-C_{22}-alkylcarbonyl radicals such as C_{10}-alkylcarbonyl, C_{11}–alkylcarbonyl, C_{12}–alkylcarbonyl, C_{13}–alkylcarbonyl, C_{14}-alkylcarbonyl, C_{16}–alkylcarbonyl, C_{18}–alkylcarbonyl, C_{20}–alkylcarbonyl or C_{22}-alkylcarbonyl radicals which comprise one or more double bonds. Particularly preferred are saturated or unsaturated C_{20}-C_{22}-alkylcarbonyl radicals such as C_{20}-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-C_{24}-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 C_{10}-C_{22}-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 C_{10}-C_{22}-alkylcarbonyl radicals such as C_{10}-alkylcarbonyl, C_{11}–alkylcarbonyl, C_{12}–alkylcarbonyl, C_{13}–alkylcarbonyl, C_{14}-alkylcarbonyl, C_{16}–alkylcarbonyl, C_{18}–alkylcarbonyl, C_{20}–alkylcarbonyl or C_{22}-alkylcarbonyl radicals which comprise one or more double bonds. Particularly preferred are saturated or unsaturated C_{20}-C_{22}-alkylcarbonyl radicals such as C_{20}-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 polyunsaturated fatty acids produced in the process 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. Fatty acids produced in the method of the present invention, preferably, comprise 20 or 22 carbon atoms in the fatty acid chain. Saturated fatty acids are ad-

vantageously 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_{20}-C_{22}-alkylcarbonyl; especially advantageously, are independently of one another unsaturated C_{20}- or C_{22}-alkylcarbonyl with at least two double bonds.

The polyunsaturated fatty acids produced by the method of 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 LCPUFAs of C_{20}- and/or C_{22}-fatty acids.

The method of the invention, advantageously, yields fatty acid esters with polyunsaturated C_{20}- and/or C_{22}-fatty acid molecules with at least two double bonds in the fatty acid ester, preferably, with at least two, three, four, five or six double bonds in the fatty acid ester, more preferably, of at least three, four, five or six double bonds in the fatty acid ester. These fatty acid esteres, preferably, lead to the synthesis of ETA, EPA and/or DHA.

The fatty acid esters with polyunsaturated C_{20}- and/or C_{22}-fatty acid molecules can be isolated in the form of an oil or lipid, for example, in the form of compounds such as sphingolipids, phosphoglycerides, lipids, glycolipids such as glycosphingolipids, phospholipids such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol or diphosphatidylglycerol, monoacylglycerides, diacylglycerides, triacylglycerides or other fatty acid esters such as the acetyl-coenzyme A esters which comprise the polyunsaturated fatty acids with at least two, three, four, five or six, preferably five or six, double bonds, from the organisms which

were used for the preparation of the fatty acid esters. Preferably, they are isolated in the form of their diacylglycerides, triacylglycerides and/or in the form of phosphatidylcholine, especially preferably in the form of the triacylglycerides. In addition to these esters, the polyunsaturated fatty acids are also present in the non-human transgenic organisms or host cells, preferably in the plants, as free fatty acids or bound in other compounds. As a rule, the various abovementioned compounds (fatty acid esters and free fatty acids) are present in the organisms with an approximate distribution of 80 to 90% by weight of triglycerides, 2 to 5% by weight of diglycerides, 5 to 10% by weight of monoglycerides, 1 to 5% by weight of free fatty acids, 2 to 8% by weight of phospholipids, the total of the various compounds amounting to 100% by weight.

In the method of the invention, the LCPUFAs which have been produced are produced in a content of at least 1 % by weight, at least 2 % by weight, at least 3% by weight, advantageously at least 5% by weight, preferably at least 8% by weight, especially preferably at least 10% by weight, very especially preferably at least 15% by weight, based on the total fatty acids in the non-human transgenic organisms or the host cell referred to above. The fatty acids are, preferably, produced in bound form. It is possible, with the aid of the polynucleotides and polypeptides of the present invention, for these unsaturated fatty acids to be positioned at the sn1, sn2 and/or sn3 position of the triglycerides which are, preferably, to be produced.

In the LCPUFA manufacturing method of the present invention the polynucleotides and polypeptides of the present invention may be used with at least one further polynucleotide encoding an enzyme of the fatty acid or lipid biosynthesis. Preferred enzymes are in this context the Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ8-desaturase, Δ5-elongase, Δ6-elongase and/or Δ9-elongase gene. These enzymes reflect the individual steps according to which the end products of the method of the present invention, for example, ETA, EPA or DHA are produced from the starting compounds linoleic acid (C18:2) or linolenic acid (C18:3). As a rule, these compounds are not generated as essentially pure products. Rather, small traces of the precursors may be also present in the end product. If, for example, both linoleic acid and linolenic acid are present in the starting organism, or the starting plant, the end products, such as ETA, EPA or DHA, are present as mixtures. The precursors should advantageously not amount to more than 20% by weight, preferably not to more than 15% by weight, more preferably, not to more than 10% by weight, most preferably not to more than 5% by weight, based on the amount of the end product in question. Advantageously, only STA, only EPA or only, more preferably, DHA, bound or as free acids, are produced as end products in

the process of the invention in a transgenic plant. If the compounds ETA, EPA and DHA are produced simultaneously, they are, preferably, produced in a ratio of at least 1:10:20 (DHA : ETA : EPA), more preferably, the ratios are 1:5:10 or 1:2:5 and, most preferably, 1:0.1:3.

Fatty acid esters or fatty acid mixtures produced by the invention, preferably, comprise 6 to 15% of palmitic acid, 1 to 6% of stearic acid, 7-85% of oleic acid, 0.5 to 8% of vaccenic acid, 0.1 to 1% of arachic acid, 7 to 25% of saturated fatty acids, 8 to 85% of monounsaturated fatty acids and 60 to 85% of polyunsaturated fatty acids, in each case based on 100% and on the total fatty acid content of the organisms. DHA as a preferred long chain polyunsaturated fatty acid is present in the fatty acid esters or fatty acid mixtures in a concentration of, preferably, at least 0.1; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8; 0.9 or 1%, based on the total fatty acid content. Moreover, the fatty acid esters or fatty acid mixtures which have been produced by the method of the invention, preferably, comprise further fatty acids selected from the group of the fatty acids erucic acid (13-docosaenoic acid), sterculic acid (9,10-methyleneoctadec-9-enoic acid), malvalic acid (8,9-methyleneheptadec-8-enoic acid), chaulmoogric acid (cyclopentenedodecanoic acid), furan fatty acid (9,12-epoxyoctadeca-9,11-dienoic acid), vernolic acid (9,10-epoxyoctadec-12-enoic acid), tariric acid (6-octadecynoic acid), 6-nonadecynoic acid, santalbic acid (t11-octadecen-9-ynoic acid), 6,9-octadecenynoic acid, pyrulic acid (t10-heptadecen-8-ynoic acid), crepenyninic acid (9-octadecen-12-ynoic acid), 13,14-dihydroorophеic acid, octadecen-13-ene-9,11-diynoic acid, petroselenic acid (cis-6-octadecenoic acid), 9c,12t-octadecadienoic acid, calendulic acid (8t10t12c-octadecatrienoic acid), catalpic acid (9t11t13c-octadecatrienoic acid), eleostearic acid (9c11t13t-octadecatrienoic acid), jacaric acid (8c10t12c-octadecatrienoic acid), punicic acid (9c11t13c-octadecatrienoic acid), parinaric acid (9c11t13t15c-octadecatetraenoic acid), pinolenic acid (all-cis-5,9,12-octadecatrienoic acid), laballenic acid (5,6-octadecadienallenic acid), ricinoleic acid (12-hydroxyoleic acid) and/or coriolic acid (13-hydroxy-9c,11t-octadecadienoic acid). The fatty acid esters or fatty acid mixtures produced by the method of the present invention, preferably, comprise less than 0.1%, based on the total fatty acids, or no butyric acid, no cholesterol, no clupanodonic acid (= docosapentaenoic acid, $C22:5^{\Delta 4,8,12,15,21}$) and no nisinic acid (tetracosahexaenoic acid, $C23:6^{\Delta 3,8,12,15,18,21}$).

By using the polynucleotides or polypeptides of the present invention in the aforementioned methods, it is envisaged that the transgenic non-human organisms or host cells provide an increase in the yield of the LCPUFAs of at least 50%, at least 80%, at least

100% or at least 150% in comparison with a reference organism or cell (i.e. a non-transgenic or non-modified cell) when compared by means of gas chromatography (GC) analysis; see Examples.

Chemically pure LCPUFAs or fatty acid compositions can also be synthesized by the method described above. To this end, the fatty acids or the fatty acid compositions are isolated from the non-human transgenic organism, host cell or culture media of host cells, for example via extraction, distillation, crystallization, chromatography or a combination of these methods. These chemically pure fatty acids or fatty acid compositions are advantageous for applications in the food industry sector, the cosmetic sector and especially the pharmacological industry sector.

Genes encoding further enzymes or proteins involved in the fatty acid or lipid metabolism can be also applied for the method of the present invention. Suitable genes are, preferably, selected from the group consisting of acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyl transferase(s), acyl-CoA:lysophospholipid acyltransferases, fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allenoxide synthases, hydroperoxide lyases or fatty acid elongase(s) are advantageously used in combination with the ω-3-desaturase. Genes selected from the group of the Δ4-desaturases, Δ5-desaturases, Δ6-desaturases, Δ8-desaturases, Δ9-desaturases, Δ12-desaturases, Δ5-elongases, Δ6-elongases or Δ9-elongases are, more preferably, used in combination with the above genes and the polynucleotide of the present invention.

The polypeptides of the invention preferentially desaturates C_{20} and C_{22}-LCPUFAs. Within the non-human transgenic organism or the host cell, these fatty acids are converted to at least 10%, 15%, 20%, 25% or 30% from the existing fatty acid pool to give the corresponding ω-3-fatty acids. Preferred substrates of the ω-3-desaturase according to the invention are the ω-6-fatty acids bound in phospholipids. Table 1 shows the preferred substrates (i.e. DGLA, ARA and DPA) and the products (i.e. ETA, EPA and DHA).

Preferably, the LCPUFAs produced by the method of the present invention are synthesized, depending on the fatty acid present in the non-human transgenic organism or host cell, which act as starting substance for the synthesis. Since biosynthetic cascades are involved, the end products in question are not present in pure form in the

organisms or host cells. Small amounts of the precursor compounds are always additionally present in the end product. These small amounts amount to less than 20% by weight, advantageously less than 15% by weight, especially advantageously less than 10% by weight, very especially advantageously less than 5, 4, 3, 2, or 1% by weight, based on the end products.

In addition to the synthesis based on endogenous precursors present in the non-human transgenic organism or host cell, the fatty acids can also be fed externally. Preferred substrates in this context are dihomo-γ-linolenic acid ($C20:3^{\Delta 8,11,14}$), arachidonic acid ($C20:4^{\Delta 5,8,11,14}$), and docosapentaenoic acid ($C22:5^{\Delta 4,7,10,13,15}$).

To increase the yield in the above-described method for the production of oils and/or triglycerides with an advantageously elevated content of polyunsaturated fatty acids, it is preferred to increase the amount of starting product for the synthesis of fatty acids; this can be achieved, for example, by introducing, into the non-human transgenic organism or host cell, a nucleic acid which encodes a polypeptide with Δ12-desaturase activity. This is particularly preferred in oil-producing non-human organisms such as oilseed rape which are high in oleic acid. Since these organisms are only low in linoleic acid (Mikoklajczak et al., Journal of the American Oil Chemical Society, 38, 1961, 678 - 681), the use of the abovementioned Δ12-desaturases for producing the starting material linoleic acid is advantageous.

In a preferred embodiment of the method of the present invention, the said method, furthermore, comprises the step of obtaining the oils, lipids or free fatty acids from the organism or the host cell. It is to be understood that in case a host cell is exploited as a source, the LCPUFAs to be manufactured can be also obtained form the culture media.

In the case of plant cells, plant tissue or plant organs, “growing” is understood as meaning, for example, the cultivation on or in a nutrient medium, or of the intact plant on or in a substrate, for example in a hydroponic culture, potting compost or on arable land.

Transgenic plants which comprise the polyunsaturated fatty acids synthesized in the method according to the invention can advantageously be marketed directly without there being any need for the oils, lipids or fatty acids synthesized to be isolated. Plants for the method according to the invention are understood as meaning intact plants and all plant parts, plant organs or plant parts such as leaf, stem, seed, root, tubers, anthers, fibers, root hairs, stalks, embryos, calli, cotelydons, petioles, harvested material,

plant tissue, reproductive tissue and cell cultures which are derived from the transgenic plant and/or can be used for bringing about the transgenic plant. In this context, the seed comprises all parts of the seed such as the seed coats, epidermal cells, seed cells, endosperm or embryonic tissue. However, the compounds produced in the method according to the invention can also be isolated from the organisms, advantageously the plants, in the form of their oils, fat, lipids and/or free fatty acids. LCPUFAs produced by this method can be harvested by harvesting the organisms either from the culture in which they grow, or from the field. This can be done via pressing or extraction of the plant parts, preferably the plant seeds. In this context, the oils, fats, lipids and/or free fatty acids can be obtained by what is known as cold-beating or cold-pressing without applying heat by pressing. To allow for greater ease of disruption of the plant parts, specifically the seeds, they are previously comminuted, steamed or roasted. The seeds which have been pretreated in this manner can subsequently be pressed or extracted with solvent such as warm hexane. The solvent is subsequently removed again. In the case of microorganisms, for example, these are harvested and then extracted directly without further processing steps, or else disrupted and then extracted via various methods with which the skilled worker is familiar. In this manner, more than 96% of the compounds produced in the process can be isolated. Thereafter, the resulting products are processed further, i.e. refined. In this process, substances such as the plant mucilages and suspended matter are first removed. What is known as desliming can be effected enzymatically or, for example, chemico-physically by addition of acid such as phosphoric acid. Thereafter, the free fatty acids are removed by treatment with a base, for example, sodium hydroxide solution. The resulting product is washed thoroughly with water to remove the alkali remaining in the product and then dried. To remove the pigment remaining in the product, the products are subjected to bleaching, for example using fuller's earth or active charcoal. At the end, the product is deodorized, for example using steam.

One embodiment of the invention are therefore oils, lipids or fatty acids or fractions thereof which have been prepared by the above-described process, especially preferably oil, lipid or a fatty acid composition which comprise LCPUFAs and originate from transgenic plants.

As described above, these oils, lipids or fatty acids, preferably, comprise 6 to 15% of palmitic acid, 1 to 6% of stearic acid, 7-85% of oleic acid, 0.5 to 8% of vaccenic acid, 0.1 to 1% of arachic acid, 7 to 25% of saturated fatty acids, 8 to 85% of monounsaturated fatty acids and 60 to 85% of polyunsaturated fatty acids, in each case based on

100% and on the total fatty acid content of the organisms. Preferred LCPUFAs present in the fatty acid esters or fatty acid mixtures is, preferably, at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1% of DHA, EPA or ETA, based on the total fatty acid content. Moreover, the fatty acid esters or fatty acid mixtures which have been produced by the process of the invention, preferably, comprise further fatty acids selected from the group of the fatty acids erucic acid (13-docosaenoic acid), sterculic acid (9,10-methyleneoctadec-9-enoic acid), malvalic acid (8,9-methyleneheptadec-8-enoic acid), chaulmoogric acid (cyclopentenedodecanoic acid), furan fatty acid (9,12-epoxyoctadeca-9,11-dienoic acid), vernonic acid (9,10-epoxyoctadec-12-enoic acid), tariric acid (6-octadecynoic acid), 6-nonadecynoic acid, santalbic acid (t11-octadecen-9-ynoic acid), 6,9-octadecenynoic acid, pyrulic acid (t10-heptadecen-8-ynoic acid), crepenyninic acid (9-octadecen-12-ynoic acid), 13,14-dihydroorophеic acid, octadecen-13-ene-9,11-diynoic acid, petroselenic acid (cis-6-octadecenoic acid), 9c,12t-octadecadienoic acid, calendulic acid (8t10t12c-octadecatrienoic acid), catalpic acid (9t11t13c-octadecatrienoic acid), eleostearic acid (9c11t13t-octadecatrienoic acid), jacaric acid (8c10t12c-octadecatrienoic acid), punicic acid (9c11t13c-octadecatrienoic acid), parinaric acid (9c11t13t15c-octadecatetraenoic acid), pinolenic acid (all-cis-5,9,12-octadecatrienoic acid), laballenic acid (5,6-octadecadienallenic acid), ricinoleic acid (12-hydroxyoleic acid) and/or coriolic acid (13-hydroxy-9c,11t-octadecadienoic acid). The fatty acid esters or fatty acid mixtures produced by the process according to the invention advantageously comprise less than 0.1%, based on the total fatty acids, or no butter butyric acid, no cholesterol, no clupanodonic acid (= dpcpsapentaenoic acid, C22:5$^{\Delta 4,8,12,15,21}$) and no nisinic acid (tetracosahexaenoic acid, C23:6 $^{\Delta 3,8,12,15,18,21}$).

The oils, lipids or fatty acids according to the invention, preferably, comprise at least 0.5%, 1%, 2%, 3%, 4% or 5%, more preferably, at least 6%, 7%, 8%, 9% or 10%, and most preferably at least 11%, 12%, 13%, 14% or 15% of ETA, EPA and/or of DHA, based on the total fatty acid content of the production organism, advantageously of a plant, especially of an oil crop such as soybean, oilseed rape, coconut, oil palm, safflower, flax, hemp, castor-oil plant, Calendula, peanut, cacao bean, sunflower or the abovementioned other monocotyledonous or dicotyledonous oil crops.

A further embodiment according to the invention is the use of the oil, lipid, fatty acids and/or the fatty acid composition in feedstuffs, foodstuffs, dietary supplies, cosmetics or pharmaceutical compositions as set forth in detail below. The oils, lipids, fatty acids or fatty acid mixtures according to the invention can be used in the manner with which the skilled worker is familiar for mixing with other oils, lipids, fatty acids or fatty acid mix-

tures of animal origin such as, for example, fish oils.

The terms “oil”, “lipid” or “fat” are understood as meaning a fatty acid mixture comprising unsaturated or saturated, preferably esterified, fatty acid(s). The oil, lipid or fat is preferably high in polyunsaturated free or, advantageously, 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 example, be determined by GC after converting the fatty acids into the methyl esters by transesterification. The oil, lipid or fat 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 polyunsaturated fatty acids with at least two double bonds, which acids are produced by the method of the present invention are, as described in detail above. They can be liberated, for example, via treatment with alkali, for example aqueous KOH or NaOH, or acid hydrolysis, preferably in the presence of an alcohol such as methanol or ethanol, or via enzymatic cleavage, and isolated via, for example, phase separation and subsequent acidification via, for example, H_2SO_4. The fatty acids can also be liberated directly without the above-described processing step.

If microorganisms are used as host cells or non-human transgenic organisms in the method of the present invention, they will be cultured, or grown, in the manner with which the skilled worker is familiar, depending on the microorganism to be used. As a rule, microorganisms will be grown in a liquid medium comprising a carbon source, mostly in the form of sugars, a nitrogen source, mostly in the form of organic nitrogen sources such as yeast extract or salts such as ammonium sulfate, trace elements such as iron, manganese and magnesium salts, and, if appropriate, vitamins, at temperatures between 0°C and 100°C, preferably between 10°C to 60°C, while gassing in oxygen. During this process, the pH of the liquid nutrient may be kept constant, i.e. regulated during the culture period, or not. The culture can be effected batchwise, semi-batchwise or continuously. Nutrients can be introduced at the beginning of the fermentation or fed in semicontinuously or continuously. The polyunsaturated fatty acids produced can be isolated from the organisms by methods with which the skilled worker is familiar, as described above; for example via extraction, distillation, crystallization, if

appropriate salt precipitation and/or chromatography. To do so, the organisms can advantageously be disrupted beforehand.

Culturing of the microorganism may be carried out at a temperature of between 0°C to 95°C, preferably between 10°C to 85°C, more preferably between 15°C to 75°C, most preferably between 15°C to 45°C.

The pH shall be maintained at between pH 4 and pH 12, preferably between pH 6 and pH 9, especially preferably between pH 7 and pH 8.

The process according to the invention can be carried out batchwise, semibatchwise or continuously. A summary of known cultivation methods is to be found in the textbook by Chmiel (Bioprozeßtechnik 1. Einführung in die Bioverfahrenstechnik (Gustav Fischer Verlag, Stuttgart, 1991)) or in the textbook by Storhas (Bioreaktoren und periphere Einrichtungen (Vieweg Verlag, Braunschweig/Wiesbaden, 1994)).

The culture medium to be used must satisfy in a suitable manner the demands of the respective strains. There are descriptions of culture media for various microorganisms in the handbook "Manual of Methods for General Bacteriology" of the American Society for Bacteriology (Washington D.C., USA, 1981).

These media which can be employed according to the invention usually comprise one or more carbon sources, nitrogen sources, inorganic salts, vitamins and/or trace elements, as described above.

Preferred carbon sources are sugars such as mono-, di- or polysaccharides. Examples of very good carbon sources are glucose, fructose, mannose, galactose, ribose, sorbose, ribulose, lactose, maltose, sucrose, raffinose, starch or cellulose. Sugars can be put in the media also via complex compounds such as molasses, or other by-products of sugar refining. It may also be advantageous to add mixtures of various carbon sources. Other possible carbon sources are oils and fats such as, for example, soybean oil, sunflower oil, peanut oil and/or coconut fat, fatty acids such as, for example, palmitic acid, stearic acid and/or linoleic acid, alcohols and/or polyalcohols such as, for example, glycerol, methanol and/or ethanol and/or organic acids such as, for example, acetic acid and/or lactic acid.

Nitrogen sources are usually organic or inorganic nitrogen compounds or materials comprising these compounds. Examples of nitrogen sources include ammonia gas, ammonia liquid or ammonium salts such as ammonium sulfate, ammonium chloride,

ammonium phosphate, ammonium carbonate or ammonium nitrate, nitrates, urea, amino acids or complex nitrogen sources such as corn steep liquor, soybean flour, soybean protein, yeast extract, meat extract and others. The nitrogen sources may be used singly or as mixtures.

Inorganic salt compounds which may be present in the media comprise the chloride, phosphoric or sulfate salts of calcium, magnesium, sodium, cobalt, molybdenum, potassium, manganese, zinc, copper and iron.

For producing sulfur-containing fine chemicals, especially methionine, it is possible to use as sulfur source inorganic sulfur-containing compounds such as, for example, sulfates, sulfites, dithionites, tetrathionates, thiosulfates, sulfides, but also organic sulfur compounds such as mercaptans and thiols.

It is possible to use as phosphorus source phosphoric acid, potassium dihydrogenphosphate or dipotassium hydrogenphosphate or the corresponding sodium-containing salts.

Chelating agents can be added to the medium in order to keep the metal ions in solution. Particularly suitable chelating agents comprise dihydroxyphenols such as catechol or protocatechuate, or organic acids such as citric acid.

The fermentation media employed according to the present invention for the culture of microorganisms normally also comprise other growth factors such as vitamins or growth promoters, which include for example biotin, riboflavin, thiamine, folic acid, nicotinic acid, pantothenate and pyridoxine. Growth factors and salts are frequently derived from complex components of the media, such as yeast extract, molasses, corn steep liquor and the like. Suitable precursors may also be added to the culture medium. The exact composition of the compounds in the media depends greatly on the particular experiment and will be decided individually for each specific case. Information on optimization of media is obtainable from the textbook ”Applied Microbiol. Physiology, A Practical Approach” (editors P.M. Rhodes, P.F. Stanbury, IRL Press (1997) pp. 53-73, ISBN 0 19 963577 3). Growth media can also be purchased from commercial suppliers, such as Standard 1 (Merck) or BHI (Brain heart infusion, DIFCO) and the like.

All the components of the media are sterilized either by heat (20 min at 1.5 bar and 121°C) or by filter sterilization. The components can be sterilized either together or, if necessary, separately. All the components of the media may be present at the start of culturing or optionally be added continuously or batchwise.

The temperature of the culture is normally between 15°C and 45°C, preferably at 25°C to 40°C, and can be kept constant or changed during the experiment. The pH of the medium should be in the range from 5 to 8.5, preferably around 7.0. The pH for the culturing can be controlled during the culturing by adding basic compounds such as sodium hydroxide, potassium hydroxide, ammonia or aqueous ammonia or acidic compounds such as phosphoric acid or sulfuric acid. The development of foam can be controlled by employing antifoams such as, for example, fatty acid polyglycol esters. The stability of plasmids can be maintained by adding to the medium suitable substances with a selective action, such as, for example, antibiotics. Aerobic conditions are maintained by introducing oxygen or oxygen-containing gas mixtures such as, for example, ambient air into the culture. The temperature of the culture is normally 20°C to 45°C, and preferably 25°C to 40°C. The culture is continued until formation of the desired product is at a maximum. This aim is normally reached within 10 hours to 160 hours.

The dry matter content of the fermentation broths obtained in this way and comprising in particular polyunsaturated fatty acids is normally from 7.5 to 25% by weight.

The fermentation broth can then be processed further. Depending on the requirement, the biomass can be removed wholly or partly from the fermentation broth by separation methods such as, for example, centrifugation, filtration, decantation or a combination of these methods, or left completely in it. The biomass is advantageously worked up after removal.

However, the fermentation broth can also be thickened or concentrated by known methods such as, for example, with the aid of a rotary evaporator, thin-film evaporator, falling-film evaporator, by reverse osmosis or by nanofiltration, without involving a cell removal step. This concentrated fermentation broth can then be worked up to obtain the fatty acids comprised therein.

The present invention, furthermore, relates to a method for the manufacture of an oil-, fatty acid- or lipid-containing composition comprising the steps of the method of the present invention and the further step or formulating the compound as an oil-, fatty acid- or lipid-containing composition.

The term “composition” refers to any composition formulated in solid, liquid or gaseous form. Said composition comprises the compound of the invention optionally together with suitable auxiliary compounds such as diluents or carriers or further ingredients. In this context, it is distinguished for the present invention between auxiliary compounds,

i.e. compounds which do not contribute to the effects elicited by the compounds of the present invention upon application of the composition for its desired purpose, and further ingredients, i.e. compounds which contribute a further effect or modulate the effect of the compounds of the present invention. Suitable diluents and/or carriers depend on the purpose for which the composition is to be used and the other ingredients. The person skilled in the art can determine such suitable diluents and/or carriers without further ado. Examples of suitable carriers and/or diluents are well known in the art and include saline solutions such as buffers, water, emulsions, such as oil/water emulsions, various types of wetting agents, etc..

In a more preferred embodiment of the oil-, fatty acid or lipid-containing composition, the said composition is further formulated as a pharmaceutical composition, a cosmetic composition, a foodstuff, a feedstuff, preferably, fish feed or a dietary supply.

The term “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 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 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 compositions 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 factors, 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(s) 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 adopted 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.

The term “cosmetic composition” relates to a composition which can be formulated as described for a pharmaceutical composition above. For a cosmetic composition, likewise, it is envisaged that the compounds of the present invention are also, preferably, used in substantially pure form. Impurities, however, may be less critical than for a pharmaceutical composition. Cosmetic compositions are, preferably, to be applied topically. Preferred cosmetic compositions comprising the compounds of the present invention 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.

Finally, as is evident from the above, the present invention, in principle, relates to the use of the polynucleotides, vectors, host cells or transgenic non-human organisms of

the present invention for the manufacture of a oil-, fatty acid- or lipid-containing composition. Preferably, the said composition is to be used as a pharmaceutical composition, cosmetic composition, foodstuff, feedstuff, preferably, fish feed or dietary supply.

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

The figures show:

Figure 1: The figure shows a comparison of the DNA sequences for the two ω-3 desaturase polynucleotides from Pythium irrgulare (SEQ ID NOS: 1 and 23).

Figure 2: The figure shows an alignment of the deduced amino acids for the two ω-3 desaturase polynucleotides from Pythium irrgulare (SEQ ID NOS: 2 and 24).

Figure 3: A comparison of the deduced amino acids for the ω-3 desaturase polypeptides from Pythium irrgulare, Phytophthora infestans and Saprolegnia declina is shown.

Figure 4: GC analysis of fatty acid methyl esters from the yeast transformant pYES2-O3 and the control pYES2 fed with GDLA.

Figure 5: GC analysis of fatty acid methyl esters from the yeast transformant pYES2-O3 and the control pYES2 fed with ARA.

Figure 6: GC analysis of fatty acid methyl esters from the yeast transformant pYES2-O3 and the control pYES2 fed with DPA (ω-6).

The following Examples shall merely illustrate the invention. They shall not be construed, whatsoever, to limit the scope of the invention.

Example 1: Isolation of novel ω-3 desaturase polynucleotides from Pythium irregulare

ω-3 desaturases are the enzymes which are able to convert ω-6 fatty acids into their corresponding ω-3 PUFAs. In order to isolate polynucleotides encoding said enzymes,

Pythium irregulare strain 10951 was ordered from ATCC. It was grown in liquid media YETG at room temperature for 5 days with constant agitation at 250 rpm. Total RNA was isolated from the harvested mycelia using TRIzol reagent (Invitrogen). The cDNA was synthesized using the Superscript III first strand kit (Invitrogen). Two pairs of degenerate primers were designed based on the conserved domains of omega-3 desaturase genes. RT-PCR was conducted to amplify the ω-3 fragments using the *Pythium* cDNA as the template by
TTYTGGGGNTTYTTYACNGT (forward primer; SEQ ID NO: 46) and
CCYTTNACYTANGTCCACT (reverse primer; SEQ ID NO: 47).
A 500 base-pair (bp) fragment was amplified and cloned into pCR4-TOPO vector (Invitrogen). A blast search from the sequence of the 500 bp fragment confirmed that it was an omega-3 desaturase gene from *Pythium irregulare*.

Based on the sequence of the ω-3 desaturase fragment from *Pythium irregulare*, two pairs of race primers and one pair of nested PCR primers were designed
(TCGCGCTCGCATGTGCTCAACTTCAG, RACE-F1, SEQ ID NO: 48; TGGTGACCACGAGCATCGTGGCGAAG, RACE-R1, SEQ ID NO: 49;
TCCTCACGCCGTTCGAGTCCTGGAAG, RACE-F1, SEQ ID NO: 50;
ATGGTCGTGAAGCCCAAGACGAAGGTC, RACE-R2, SEQ ID NO: 51).
A Marathon RACE cDNA library (BD Biosciences) was made using the messenger RNA isolated from total RNA from *Pythium irregulare*. PCR reactions for 3' and 5' races were applied to amplify a 800 bp and a 1000 bp fragments, respectively, from 3' and 5' RACE. These fragments were cloned into pCR4-TOPO vector (Invitrogen). Four positive clones from each race were sequenced and there are some variations among them. Therefore *Pythium irregulare* may have more than one ω-3 desaturase genes.

The assembled ω-3 desaturase gene contains a 1092 bp of open reading frame. Based the assembled ω-3 desaturase gene, one pair of primers
(TCCGCTCGCCATGGCGTCCAC, O3-Yes1, SEQ ID NO: 52 and TGACCGATCACTTAGCTGCAGCTTA, O3-Yes2, SEQ ID NO: 53)
was designed to amplify the full length of O3 genes (ω-3 desaturase genes) from *Pythium*. The full length O3 from Pythium was cloned into yeast expression vector pYES2.1/V5-His-TOPO. Eight of full length clones were sequenced. Six of them are identical. This gene was designated as O3-Pythiym1. Two of other ones are identical, which was designated as O3-Pythgium2. Two genes are 99% identical (Figure 1) and they only have one amino acid different (Figure 2). The O3 desaturase protein from Pythium is 69% and 60% identical to ω-3 desaturase from *P. infestans* (WO

2005/083053) and ω-3 desaturase gene from *Saprolegnia diclina* (WO 2004/071467) (Figure 3), respectively. It has low identities to delta-12 and delta-15 desaturase genes.

Example 2: Characterization of novel ω-3 desaturases from Pythium irregulare

The plasmids containing the full length O3 genes in the yeast expression vector pYES2.1/V5-His-TOPO were transformed into yeast *S. cerevisiae*. The positive transformants were selected for uracil auxotrophy on DOB-U agar plates. To characterize the ω-3 desaturase enzyme activity, positive clones and the control (yeast with pYES2.1 vector) were cultured overnight in DOB-U liquid medium at 28°C and then grown in induction medium (DOB-U+Gal+Raf) containing 100 μM of various exogenously supplied fatty acid substrates at 16°C for 4 days. The whole yeast cells expressing Pythium ω-3 genes were harvested by centrifugation and washed twice with distilled water. Then the yeast cells were directly transmethylated with methanolic HCl (3N) at 80°C for 1 hour. The resultant methyl esters were extracted with hexane and analyzed by gas chromatography (GC). GC was carried out as described in WO 2005/083053.

The expression results showed that ω-3 desaturase from Pythium is not able to desaturase the 18-carbon ω-6 fatty acids, such as LA and GLA. It desaturates the ω-6 fatty acids longer than 18-carbon chains, such as DGLA (Figure 4), ARA (Figure 5) and DPA (Figure 6). However, it is more specific to ARA with over 40% conversion rate (Table 1).

Table 1: Production of ω-3 fatty acids from exogenous ω-6 fatty acids in the yeast transformant (pYES2-O3) and the control yeast pYES2

Substrate	**Substrate (%)**	**Product**	**Product (%)**	**Conversion (%)**
pYES2				
LA	24.50	**ALA**	0	
GLA	21.97	**SDA**	0	
DGLA	15.79	**ETA**	0	
ARA	6.45	**EPA**	0	
DPA	8.26	**DHA**	0.03	

pYES2-O3				
LA	25.56	**ALA**	0	0
GLA	22.79	**SDA**	0	0
DGLA	17.78	**ETA**	1.90	9.65 %
ARA	7.19	**EPA**	4.95	40.77 %
DPA	9.52	**DHA**	0.20	2.01 %

In summary, two ω -3 desaturase isoforms were isolated from *Pythium irregulare* and both are able to introduce an ω-3 double bond into ω-6 fatty acids longer than 18 carbon chains supplied exogenously in yeast. Moreover, this is apparently the first ω-3 desaturase that is able to convert the ω-6 DPA into DHA.

Patent claims

1. A polynucleotide comprising a nucleic acid sequences selected from the group consisting of:

   (a) a nucleic acid sequence as shown in SEQ ID No. 1 or 23;

   (b) a nucleic acid sequence encoding a polypeptide having an amino acid sequence as shown in SEQ ID No. 2 or 24;

   (c) a nucleic acid sequence which is at least 70% identical to the nucleic acid sequence of (a) or (b), wherein said nucleic acid sequence encodes a polypeptide having omega -3 desaturase activity;

   (d) a nucleic acid sequence being a fragment of any one of (a) to (c), wherein said fragment encodes a polypeptide having omega-3 desaturase activity; and

   (e) a nucleic acid sequence encoding a polypeptide having ω-3 desaturase activity, wherein saif polypeptide comprises a polypeptide pattern as shown in a sequence selected from the group consisting of SEQ ID NO: 15, 16, 17, 18, 19, 20, 21, 22, 37, 38, 39, 40, 41, 42, 43, 44 and 45.

2. The polynucleotide of claim 1, wherein said polynucleotide is DNA or RNA.

3. A vector comprising the polynucleotide of claim 1 or 2.

4. The vector of claim 3, wherein said vector is an expression vector.

5. The vector of claim 3 or 4, wherein said vector comprises at least one polynucleotide encoding a further enzyme being involved in the biosynthesis of fatty acids or lipids.

6. A host cell comprising the polynucleotide of claim 1 or 2 the vector of any one of claims 3 to 5.

7. The host cell of claim 6, wherein said host cell additionally comprises at least one further enzyme being involved in the biosynthesis of fatty acids or lipids.

8. The vector of claim 5 or the host cell of claim 7, wherein said further enzyme is selected from the groups consisting of: acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyltransferase(s), acyl-CoA:lysophospholipid acyltransferase(s), fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenase(s), lipoxygenase(s), triacylglycerol lipase(s), allenoxide synthase(s), hydroperoxide lyase(s) or fatty acid elongase(s), acyl-CoA:lysophospholipid acyltransferase, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ8-desaturase, Δ9-desaturase, Δ12-desaturase, Δ5-elongase, Δ6-elongase and Δ9-elongase.

9. A method for the manufacture of a polypeptide having ω-3 desaturase activity comprising:
   (a) expressing the polynucleotide of claim 1 or 2 in a host cell; and
   (b) obtaining the polypeptide encoded by said polynucleotide from the host cell.

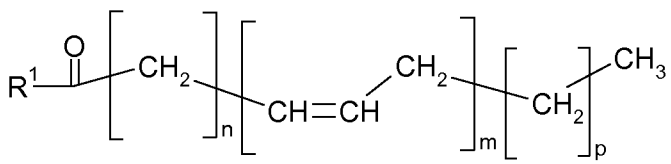
10. A polypeptide encoded by the polynucleotide of claim 1 or 2 or which is obtainable by the method of claim 9.

11. An antibody which specifically recognizes the polypeptide of claim 10.

12. A transgenic non-human organism comprising the polynucleotide of claim 1 or 2, the vector of any one of claims 3 to 5, the host cell of claim 6 or 7 or the vector or host cell of claim 8.

13. The transgenic non-human organism of claim 12, wherein said organism is an animal, a plant or a multicellular micro-organism.

14. A method for the manufacture of a compound having a structure as shown in the general formula I

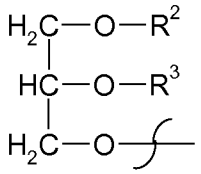


(I)

wherein the variables and substituents in formula I are

R^1 = hydroxyl, coenzyme A (thioester), lysophosphatidylcholine, lysophos-

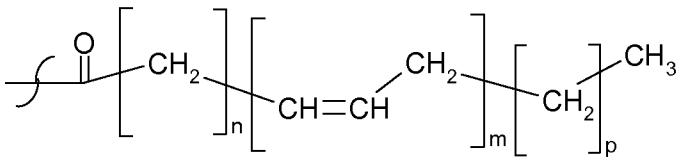
phatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidylglycerol, lysophosphatidylserine, lysophosphatidylinositol, sphingo base or a radical of the formula II



(II),

R^2 = hydrogen, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidylglycerol, lysophosphatidylserine, lysophosphatidylinositol or saturated or unsaturated C_2-C_{24}-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:



(Ia)

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

and

wherein said method comprises cultivating (i) the host cell of any of claims 3 to 5, (ii) the transgenic non-human organism of claim 12 or 13 or (iii) a host cell or a transgenic non-human organism comprising a polynucleotide comprising a nucleic acid sequence as shown in any one of SEQ ID NOs: 6, 7, 9, 11, 13, 30, 33 or 35 or which encodes a polypeptide having an amino acid sequence as shown in any one of SEQ ID NOs: 8, 10, 12, 14, 31, 34 or 36 under conditions which allow biosynthesis of the said compound.

15. A method for the manufacture of an oil-, fatty acid- or lipid-containing composition comprising the steps of the method of claim 14 and the further step or formulating the compound as an oil-, fatty acid- or lipid-containing composition.

16. The method of claim 15, wherein said oil-, fatty acid or lipid-containing composition is further formulated as a pharmaceutical composition, a cosmetic composition, a foodstuff, a feedstuff, a fish feed or a dietary supply.

17. Use of the polynucleotide of claim 1 or 2, the vector of any one of claims 3 to 5, the host cell of claims 6 or 7, the vector or host cell of claim 8, the polypeptide of claim 10 or the transgenic non-human organism of claim 12 or 13 for the manufacture of an oil-, fatty acid- or lipid-containing composition.

18. The use of claim 17, wherein said oil-, fatty acid- or lipid-containing composition is to be used as a pharmaceutical composition, cosmetic composition, foodstuff, feedstuff, fish feed or dietary supply.

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                    1                                                                                   85
O3-Pythium1      (1) ATGGCGTCCACCTCCGCCGCCCAAGACGCCGCGCCGTACGAGTTCCCGTCGCTGACCGAGATCAAGCGCGCGCTGCCCAGCGAGT
O3-Pythium2      (1) ATGGCGTCCACCTCCGCCGCCCAAGACGCCGCGCCGTACGAGTTCCCGTCGCTGACCGAGATCAAGCGCGCGCTGCCCAGCGAGT
                    86                                                                                 170
O3-Pythium1     (86) GCTTCGAGGCGTCGGTGCCCCTGTCGCTCTACTACACCGCGCGCTCGCTGGCGCTTGCCGGCTCGCTCGCGGTCGCGCTCTCGTA
O3-Pythium2     (86) GCTTCGAAGCGTCGGTGCCCCTGTCGCTCTACTACACCGCGCGCTCGCTGGCGCTTGCCGGCTCGCTCGCGGTCGCGCTCTCGTA
                    171                                                                                255
O3-Pythium1    (171) TGCGCGTGCGCTGCCGCTGGTGCAGGCGAACGCGCTGCTCGACGCCACGCTCTGTACGGGCTACGTGCTACTGCAGGGCATCGTG
O3-Pythium2    (171) CGCGCGTGCGCTGCCGCTGGTGCAGGCGAACGCGCTGCTCGACGCCACGCTCTGTACGGGCTACGTGCTACTGCAGGGCATCGTG
                    256                                                                                340
O3-Pythium1    (256) TTCTGGGGCTTCTTCACAGTCGGCCACGACTGCGGCCACGGCGCGTTCTCGCGCTCGCATGTGCTCAACTTCAGCGTCGGCACGC
O3-Pythium2    (256) TTCTGGGGCTTCTTCACAGTCGGCCACGACTGCGGCCACGGCGCGTTCTCGCGCTCGCATGTGCTCAACTTCAGCGTCGGCACGC
                    341                                                                                425
O3-Pythium1    (341) TCATGCACTCGATCATCCTCACGCCGTTCGAGTCCTGGAAGCTGTCGCACCGCCACCACCACAAGAACACGGGCAACATCGACAA
O3-Pythium2    (341) TCATGCACTCGATCATCCTCACGCCGTTCGAGTCCTGGAAGCTGTCGCACCGCCACCACCACAAGAACACGGGCAACATCGACAA
                    426                                                                                510
O3-Pythium1    (426) GGACGAGATCTTCTACCCGCAGCGCGAGGCTGACTCACACCCAGTCTCCCGCCACTTGGTCATGTCGCTCGGCTCGGCGTGGTTT
O3-Pythium2    (426) GGACGAGATCTTCTACCCGCAGCGCGAGGCTGACTCGCACCCAGTCTCCCGCCACTTGGTCATGTCGCTCGGCTCGGCGTGGTTT
                    511                                                                                595
O3-Pythium1    (511) GCCTACCTGTTCGCGGGCTTCCCTCCTCGCACGATGAACCACTTCAACCCGTGGGAAGCGATGTACGTGCGTCGTGTGGCCGCTG
O3-Pythium2    (511) GCCTACCTGTTCGCGGGCTTCCCTCCTCGCACGATGAACCACTTCAACCCGTGGGAAGCGATGTACGTGCGTCGTGTGGCCGCTG
                    596                                                                                680
O3-Pythium1    (596) TGATCATCTCGCTCGGCGTGCTCTTCGCCTTCGCAGGTCTCTACTCGTACTTGACCTTCGTCTTGGGCTTCACGACCATGGCGAT
O3-Pythium2    (596) TGATCATCTCGCTCGGCGTGCTCTTCGCCTTCGCAGGTCTCTACTCGTACCTGACCTTCGTCTTGGGCTTCACGACCATGGCGAT
                    681                                                                                765
O3-Pythium1    (681) CTACTACTTTGGTCCATTGTTCATCTTCGCCACGATGCTCGTGGTCACCACGTTCTTGCACCACAACGACGAAGAGACTCCGTGG
O3-Pythium2    (681) CTACTACTTTGGTCCATTGTTCGTCTTCGCCACGATGCTCGTGGTCACCACGTTCTTGCACCACAACGACGAAGAGACCCCGTGG
                    766                                                                                850
O3-Pythium1    (766) TACGCGGATTCGGAGTGGACGTACGTCAAGGGCAACCTCTCGTCCGTGGACCGCTCATACGGCGCGCTGATCGACAACCTGAGCC
O3-Pythium2    (766) TACGCGGACTCGGAGTGGACGTACGTCAAGGGCAACCTCTCGTCCGTGGACCGCTCATACGGCGCGCTGATTGACAACCTGAGCC
                    851                                                                                935
O3-Pythium1    (851) ACAACATCGGCACGCACCAGATCCACCACCTGTTCCCGATCATCCCGCACTACAAGCTCAACGACGCCACGGCGGCGTTTGCCAA
O3-Pythium2    (851) ACAACATCGGCACGCACCAGATCCACCACCTGTTCCCGATCATCCCGCACTACAAGCTCAACGACGCCACGGCGGCGTTTGCCAA
                    936                                                                               1020
O3-Pythium1    (936) GGCGTTCCCAGAGCTCGTGCGCAAGAACGCGGCGCCGATCATCCCGACGTTCTTCCGCATGGCCGCCATGTACGCCAAGTACGGC
O3-Pythium2    (936) GGCGTTCCCAGAGCTCGTGCGCAAGAACGCGGCGCCGATCATCCCGACGTTCTTCCGCATGGCCGCCATGTACGCCAAGTACGGC
                    1021                                                                           1092
O3-Pythium1   (1021) GTGGTCGACACGGACGCCAAGACGTTCACGCTCAAGGAAGCCAAGGCCGCCGCCAAGACCAAGTCGAGCTAA
O3-Pythium2   (1021) GTGGTCGACACGGACGCCAAGACGTTCACGCTCAAGGAAGCCAAGGCCGCCGCCAAGACCAAGTCGAGCTAA
```

Figure 1

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                    1                                                                   75
O3-Pythium-1     (1) MASTSAAQDAAPYEFPSLTEIKRALPSECFEASVPLSLYYTARSLALAGSLAVALSYARALPLVQANALLDATLC
O3-Pythium-2     (1) MASTSAAQDAAPYEFPSLTEIKRALPSECFEASVPLSLYYTARSLALAGSLAVALSYARALPLVQANALLDATLC
                    76                                                                 150
O3-Pythium-1    (76) TGYVLLQGIVFWGFFTVGHDCGHGAFSRSHVLNFSVGTLMHSIILTPFESWKLSHRHHHKNTGNIDKDEIFYPQR
O3-Pythium-2    (76) TGYVLLQGIVFWGFFTVGHDCGHGAFSRSHVLNFSVGTLMHSIILTPFESWKLSHRHHHKNTGNIDKDEIFYPQR
                    151                                                                225
O3-Pythium-1   (151) EADSHPVSRHLVMSLGSAWFAYLFAGFPPRTMNHFNPWEAMYVRRVAAVIISLGVLFAFAGLYSYLTFVLGFTTM
O3-Pythium-2   (151) EADSHPVSRHLVMSLGSAWFAYLFAGFPPRTMNHFNPWEAMYVRRVAAVIISLGVLFAFAGLYSYLTFVLGFTTM
                    226                                                                300
O3-Pythium-1   (226) AIYYFGPLFIFATMLVVTTFLHHNDEETPWYADSEWTYVKGNLSSVDRSYGALIDNLSHNIGTHQIHHLFPIIPH
O3-Pythium-2   (226) AIYYFGPLFVFATMLVVTTFLHHNDEETPWYADSEWTYVKGNLSSVDRSYGALIDNLSHNIGTHQIHHLFPIIPH
                    301                                                    363
O3-Pythium-1   (301) YKLNDATAAFAKAFPELVRKNAAPIIPTFFRMAAMYAKYGVVDTDAKTFTLKEAKAAAKTKSS
O3-Pythium-2   (301) YKLNDATAAFAKAFPELVRKNAAPIIPTFFRMAAMYAKYGVVDTDAKTFTLKEAKAAAKTKSS
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Figure 2

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                        1                                                                   75
O3-P.irregulare     (1) MASTSAAQDAAPYEFPSLTEIKRALPSECFEASVPLSLYYTARSLALAGSLAVALSYARALPLVQANALLDATLC
 O3-P.infestans     (1) ------MATKEAYVFPTLTEIKRSLPKDCFEASVPLSLYYTVRCLVIAVALTFGLNYARALPEVESFWALDAALC
   O3-S.diclina     (1) -----MTEDKTKVEFPTLTELKHSIPNACFESNLGLSLYYTARAIFNASASAALLYAARSTPFIADNVLLHALVC
                        76                                                                 150
O3-P.irregulare    (76) TGYVLLQGIVFWGFFTVGHDCGHGAFSRSHVLNFSVGTLMHSIILTPFESWKLSHRHHHKNTGNIDKDEIFYPQR
 O3-P.infestans    (70) TGYILLQGIVFWGFFTVGHDAGHGAFSRYHLLNFVVGTFMHSLILTPFESWKLTHRHHHKNTGNIDRDEVFYPQR
   O3-S.diclina    (71) ATYIYVQGVIFWGFFTVGHDCGHSAFSRYHSVNFIIGCIMHSAILTPFESWRVTHRHHHKNTGNIDKDEIFYPHR
                        151                                                                225
O3-P.irregulare   (151) EADSHPVSRHLVMSLGSAWFAYLFAGFPPRTMNHFNPWEAMYVRRVAAVIISLGVLFAFAGLYSYLTFVLGFTTM
 O3-P.infestans   (145) KADDHPLSRNLILALGAAWLAYLVEGFPPRKVNHFNPFEPLFVRQVSAVVISLLAHFFVAGLSIYLSLQLGLKTM
   O3-S.diclina   (146) SVKDLQDVRQWVYTLGGAWFVYLKVGYAPRTMSHFDPWDPLLLRRASAVIVSLGVWAAFFAAYAYLTYSLGFAVM
                        226                                                                300
O3-P.irregulare   (226) AIYYFGPLFVFATMLVVTTFLHHNDEETPWYADSEWTYVKGNLSSVDRSYGALIDNLSHNIGTHQIHHLFPIIPH
 O3-P.infestans   (220) AIYYYGPVFVFGSMLVITTFLHHNDEETPWYADSEWTYVKGNLSSVDRSYGALIDNLSHNIGTHQIHHLFPIIPH
   O3-S.diclina   (221) GLYYYAPLFVFASFLVITTFLHHNDEATPWYGDSEWTYVKGNLSSVDRSYGAFVDNLSHHIGTHQVHHLFPIIPH
                        301                                                            368
O3-P.irregulare   (301) YKLNDATAAFAKAFPELVRKNAAPIIPTFFRMAAMYAKYGVVDTDAKTFTLKEAKAAAKTKSS-----
 O3-P.infestans   (295) YKLKKATAAFHQAFPELVRKSDEPIIKAFFRVGRLYANYGVVDQEAKLFTLKEAKAATEAAAKTKST-
   O3-S.diclina   (296) YKLNEATKHFAAAYPHLVRRNDEPIITAFFKTAHLFVNYGAVPETAQIFTLKESAAAAKAKSD-----
```

Figure 3

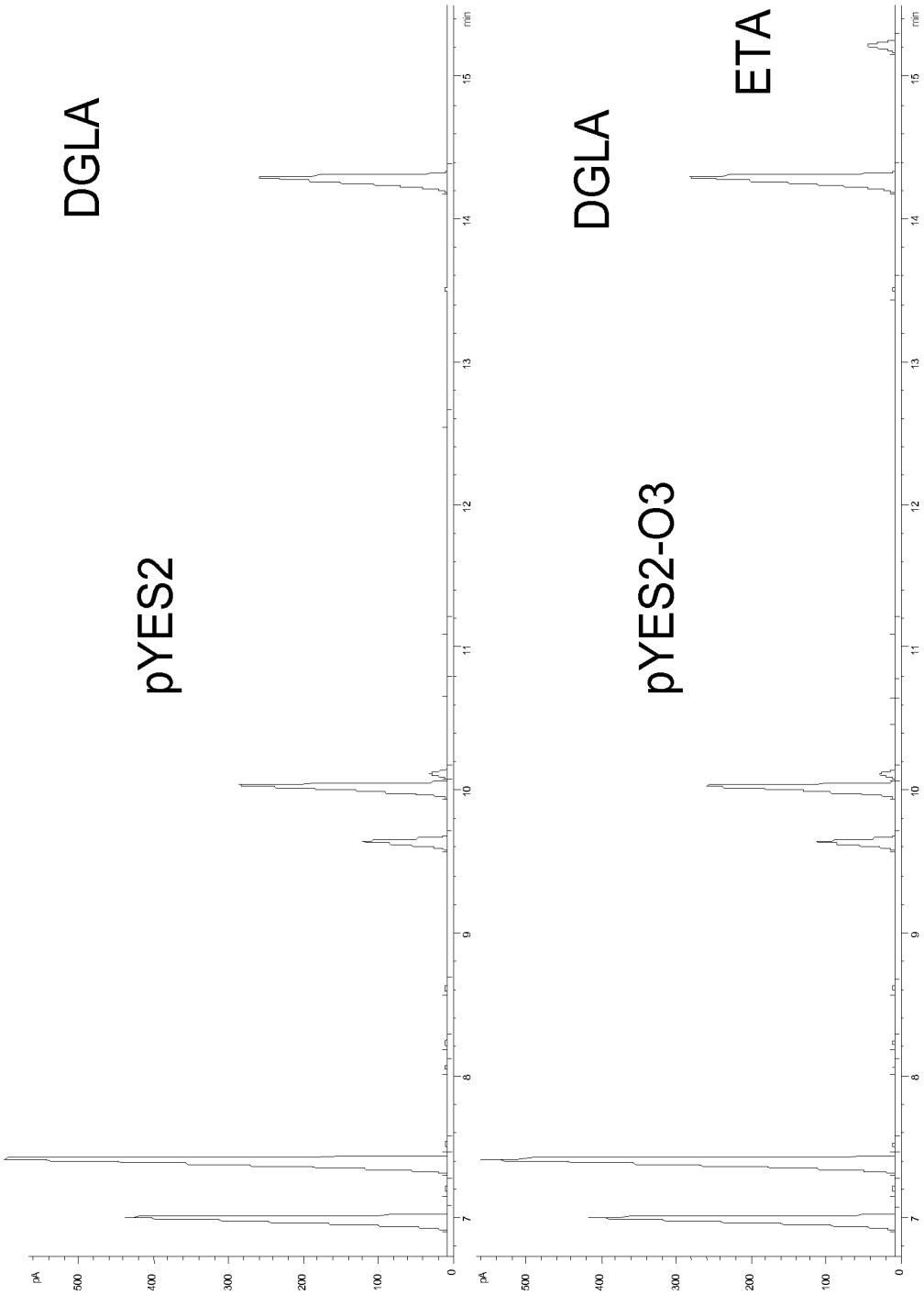
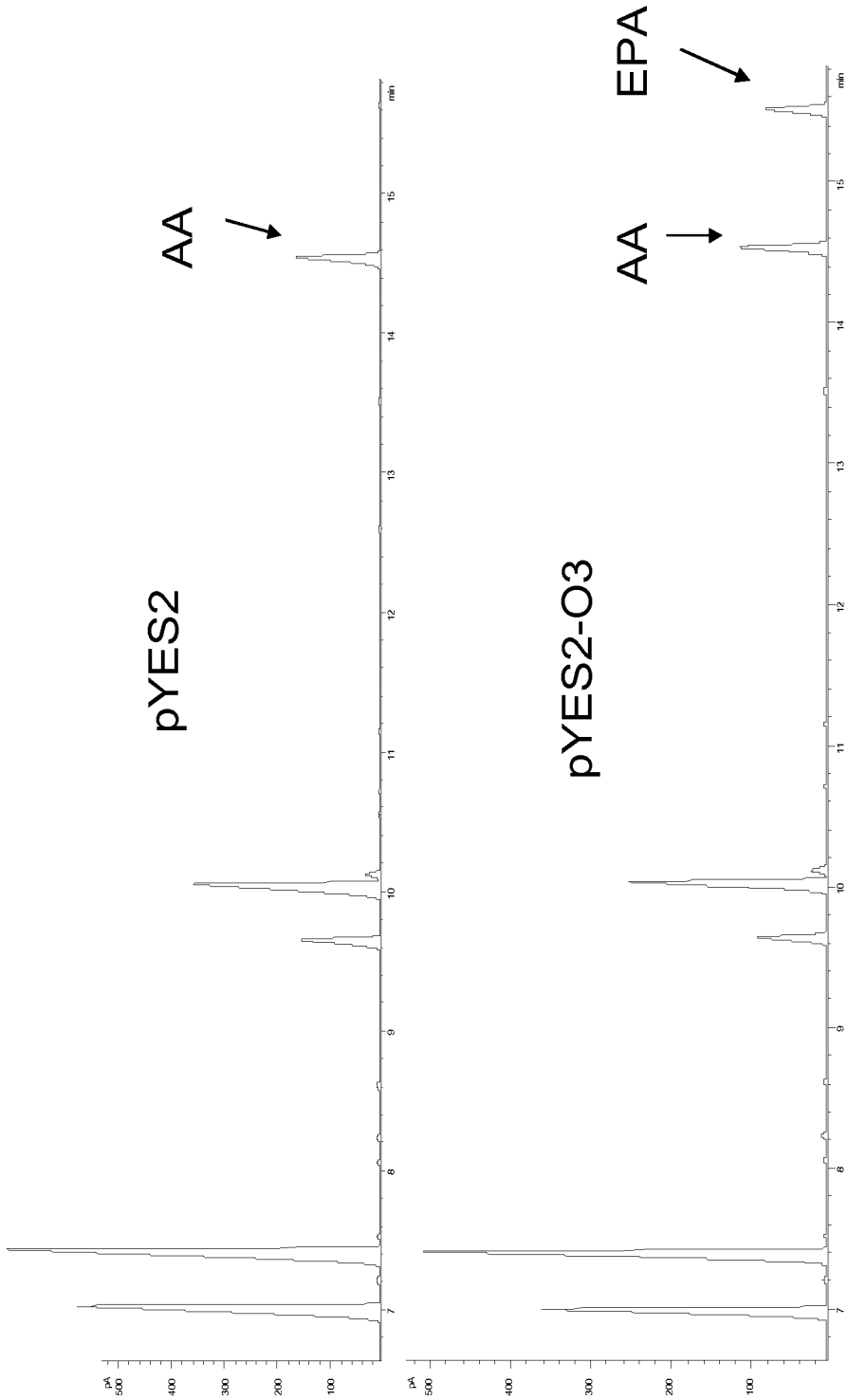

pYES2
DGLA
pYES2-O3
DGLA
ETA
pA
500
400
300
200
100
0
7
8
9
10
11
12
13
14
15
min

Figure 4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AA
pYES2-O3
AA
EPA
pA
500
400
300
200
100
min
7
8
9
10
11
12
13
14
15

Figure 5

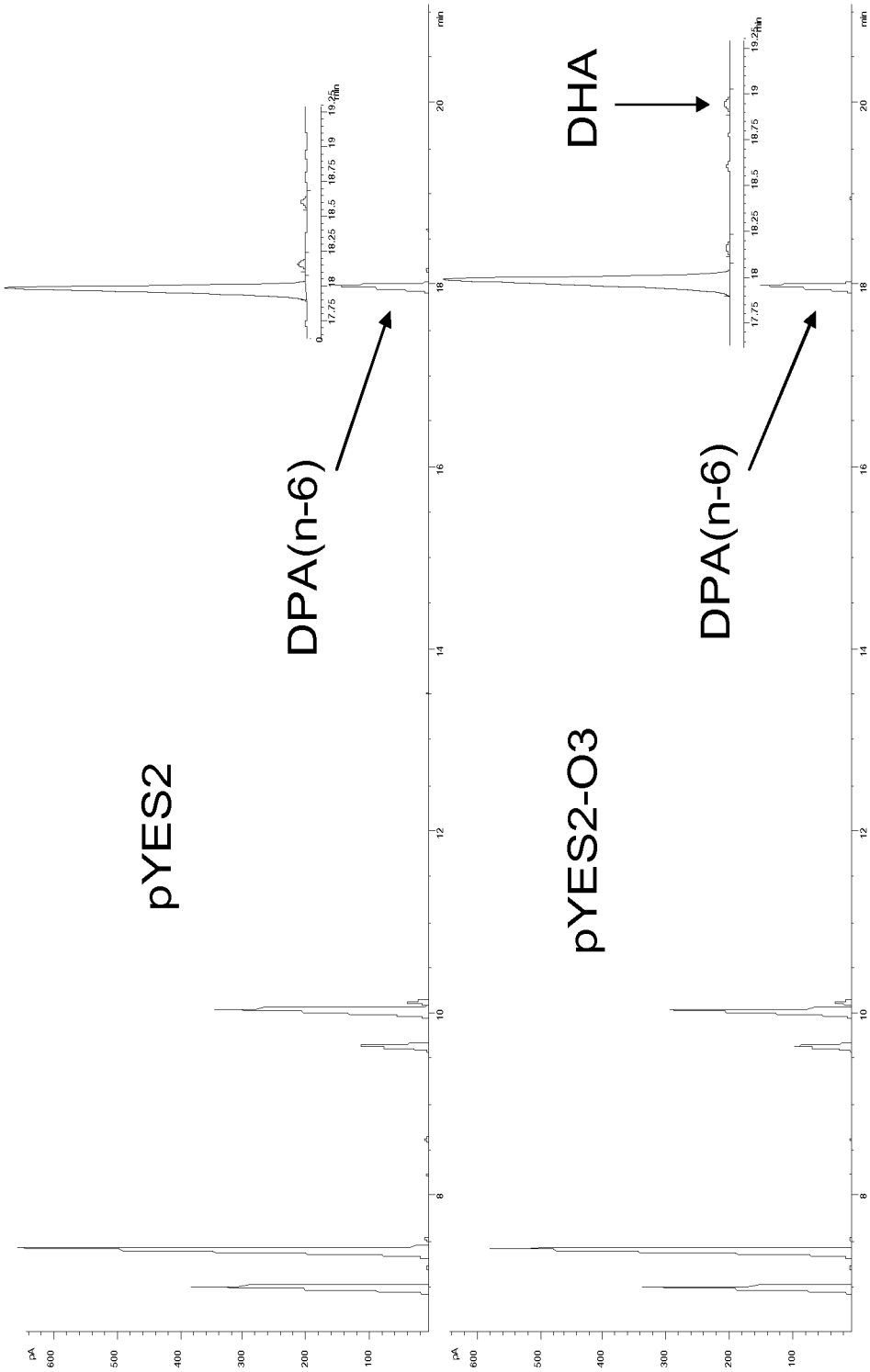

Figure 6