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(54) ACYLTRANSFERASES AND USES THEROF IN FATTY ACID PRODUCTION

ACYLTRANSFERASEN UND DEREN VERWENDUNG IN DER FETTSÄURENHERSTELLUNG

ACYLTRANSFERASES ET SES UTILISATION DANS LA PRODUCTION DES ACIDES GRAS

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• **C. Zhang ET AL: "A thraustochytrid diacylglycerol acyltransferase 2 with broad substrate specificity strongly increases oleic acid content in engineered Arabidopsis thaliana seeds", Journal of Experimental Botany, vol. 64, no. 11, 1 August 2013 (2013-08-01), pages 3189-3200, XP055082800, ISSN: 0022-0957, DOI: 10.1093/jxb/ert156**

• **IIDA I ET AL: "Improvement of docosahexaenoic acid production in a culture of Thraustochytrium aureum by medium optimization", JOURNAL OF FERMENTATION AND BIOENGINEERING, SOCIETY OF FERMENTATION TECHNOLOGY, JP, vol. 81, no. 1, 1 January 1996 (1996-01-01), pages 76-78, XP002327281, ISSN: 0922-338X, DOI: 10.1016/0922-338X(96)83125-4**

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EP 2 585 603 B1

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Description

[0001] The present invention relates to the recombinant manufacture of polyunsaturated fatty acids. Specifically, it relates to acyltransferase polypeptides, polynucleotides encoding said acyltransferase polypeptides as well to vectors, host cells, non-human transgenic organisms containing said polynucleotides. Moreover, the present invention contemplates methods for the manufacture of polyunsaturated fatty acids as well as oils obtained by such methods.

[0002] Fatty acids and triacylglycerides have a various applications in the food industry, in animal feed, supplement nutrition, and in the cosmetic and pharmacological and pharmaceutical field. The individual applications may either require free fatty acids or triacylglycerides. In both cases, however, polyunsaturated fatty acids either free or esterified are of pivotal interest for many of the aforementioned applications. In particular, polyunsaturated omega-3-fatty acids and omega-6-fatty acids are important constituents in animal and human food. These fatty acids are supposed to have beneficial effects on the overall health and, in particular, on the central nervous system, the cardiovascular system, the immune system, and the general metabolism. Within traditional food, the polyunsaturated omega-3-fatty acids are mainly found in fish and plant oils. However, in comparison with the needs of the industry and the need for a beneficial diet, this source is rather limited.

[0003] The various polyunsaturated fatty acids (PUFA) and PUFA-containing triglycerides are also mainly obtained from microorganisms such as *Mortierella* and *Schizochytrium* or from oil-producing plants such as soybean or oilseed rape, algae such as *Cryptocodinium* or *Phaeodactylum* and others, where they are usually obtained in the form of their triacylglycerides. The free PUFA are usually prepared from the triacylglycerides by hydrolysis. However, long chain polyunsaturated fatty acids (LCPUFA) having a C-18, C-20, C-22 or C-24 fatty acid body, such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), arachidonic acid (ARA), dihomo-gamma-linolenic acid or docosapentaenoic acid (DPA) can not be efficiently isolated from natural oil crop plants such as oilseed rape, soybean, sunflower or safflower. Conventional natural sources of these fatty acids are, thus, merely fish, such as herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna, or from algae.

[0004] Especially suitable microorganisms for the production of PUFA in industrial scale are microalgae such as *Phaeodactylum tricornutum*, *Porphyridium* species, *Thraustochytrium* species, *Nannochloropsis* species, *Schizochytrium* species or *Cryptocodinium* species, ciliates such as *Stylonychia* or *Colpidium*, fungi such as *Mortierella*, *Entomophthora* or *Mucor* and/or mosses such as *Physcomitrella*, *Ceratodon* and *Marchantia* (Vazhappilly 1998, *Botanica Marina* 41: 553-558; Totani 1987, *Lipids* 22: 1060-1062; Akimoto 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 PUFA. However, the mutation and selection of strains with an improved production of a particular molecule such as the polyunsaturated fatty acids is a time-consuming and difficult process. This is why recombinant methods as described above are preferred whenever possible. However, only limited amounts of the desired PUFA or LCPUFA and, in particular, DHA or EPA, can be produced with the aid of the above mentioned microorganisms, and, depending on the microorganism used, these are generally obtained as fatty acid mixtures of, for example, EPA, DPA and DHA.

[0005] Many attempts in the past have been made to make available genes which are involved in the synthesis of fatty acids or triglycerides for the production of oils in various organisms. Various desaturases have been described in the art; see, e.g., documents WO 91/13972, WO 93/11245, WO 94/11516, EP-A-0 550 162, WO 94/18337, WO 97/30582, WO 97/21340, WO 95/18222, EP-A-0 794 250, Stukey 1990, *J. Biol. Chem.*, 265: 20144-20149, Wada 1990, *Nature* 347: 200-203, Huang 1999, *Lipids* 34: 649-659, WO 93/06712, US 5,614,393, WO 96/21022, WO 00/21557, WO 99/27111, WO 98/46763, WO 98/46764, WO 98/46765, WO 99/64616 or WO 98/46776. These enzymes can be used for the production of unsaturated fatty acids. Thus, due to modern molecular biology, it has become possible to increase at least to some extent the content of the desired polyunsaturated fatty acids and, in particular, the PUFA or LCPUFA in a given organism. Elongases for the production of fatty acids are disclosed in the document WO2009/016202.

[0006] The biosynthesis of LCPUFA and the incorporation of LCPUFA into membrane lipids or triacylglycerides proceeds via various metabolic pathways (Abadi 2001, *European Journal of Lipid Science & Technology* 103:106-113). In bacteria such as *Vibrio*, and microalgae, such as *Schizochytrium*, malonyl-CoA is converted into LCPUFA via an LCPUFA-producing polyketide synthase (Metz 2001, *Science* 293: 290-293; WO 00/42195; WO 98/27203; WO 98/55625). In microalgae, such as *Phaeodactylum*, and mosses, such as *Physcomitrella*, unsaturated fatty acids such as linoleic acid or linolenic acid are converted in a plurality of desaturation and elongation steps to give LCPUFA (Zank 2000, *Biochemical Society Transactions* 28: 654-658). Desaturation takes place either on acyl groups bound to Coenzyme A (acyl-CoA) or on acyl groups of membrane lipids, whereas elongation is biochemically restricted to acyl chains bound to CoA. In mammals, the biosynthesis of DHA comprises a chain shortening via beta-oxidation, in addition to desaturation and elongation steps. In microorganisms and lower plants, LCPUFA are present either exclusively in the form of membrane lipids, as is the case in *Physcomitrella* and *Phaeodactylum*, or in membrane lipids and triacylglycerides, as is the case in *Schizochytrium* and *Mortierella*. Incorporation of LCPUFA into lipids and oils, as well as the transfer of the fatty acid moiety (acyl group) between lipids and other molecular species such as acyl-CoA, is catalyzed by various acyl-

transferases and transacylases. These enzymes are, known to carry out the incorporation or interexchange of saturated and unsaturated fatty acids (Slabas 2001, J. Plant Physiology 158: 505-513, Frentzen 1998, Fett/Lipid 100: 161-166, Cases 1998, Proc. Nat. Acad. Sci. USA 95: 13018-13023). One group of acyltransferases having three distinct enzymatic activities are enzymes of the "Kennedy pathway", which are located on the cytoplasmic side of the membrane system of the endoplasmic reticulum (ER). The ER-bound acyltransferases in the microsomal fraction use acyl-CoA as the activated form of fatty acids. Glycerol-3-phosphate acyltransferase (GPAT) catalyzes the incorporation of acyl groups at the sn-1 position of glycerol-3-phosphate. 1-Acylglycerol-3-phosphate acyltransferase, also known as lysophosphatidic acid acyltransferase (LPAAT), catalyze the incorporation of acyl groups at the sn-2 position of lysophosphatidic acid (LPA). After dephosphorylation of phosphatidic acid by phosphatidic acid phosphatase (PAP), diacylglycerol acyltransferase (DGAT) catalyzes the incorporation of acyl groups at the sn-3 position of diacylglycerols. Further enzymes directly involved in TAG biosynthesis - apart from the said Kennedy pathway enzymes - are the phospholipid diacylglycerol acyltransferase (PDAT), an enzyme that transfers acyl groups from the sn-2 position of membrane lipids to the sn-3 position of diacylglycerols, and diacylglyceroldiacylglycerol transacylase (DDAT), an enzyme that transfers acylgroups from the sn-2 position of one diacylglycerol-molecule to the sn-3 position of another diacylglycerol-molecule. Lysophospholipid acyltransferase (LPLAT) represents a class of acyltransferases that are capable of incorporating activated acyl groups from acyl-CoA to membrane lipids, and possibly catalyze also the reverse reaction. More specifically, LPLATs can have activity as lysophosphatidylethanolamine acyltransferase (LPEAT) and lysophosphatidylcholine acyltransferase (LPCAT). Further enzymes, such as lecithin cholesterol acyltransferase (LCAT) can be involved in the transfer of acyl groups from membrane lipids into triacylglycerides, as well.

[0007] The documents WO 98/54302 and WO 98/54303 disclose a human LPAAT and its potential use for the therapy of diseases, as a diagnostic, and a method for identifying modulators of the human LPAAT. Moreover, a variety of acyltransferases with a wide range of enzymatic functions have been described in the documents WO 98/55632, WO 98/55631, WO 94/13814, WO 96/24674, WO 95/27791, WO 00/18889, WO 00/18889, WO 93/10241, Akermoun 2000, Biochemical Society Transactions 28: 713-715, Tumaney 1999, Biochimica et Biophysica Acta 1439: 47-56, Fraser 2000, Biochemical Society Transactions 28: 715-7718, Stymne 1984, Biochem. J. 223: 305-314, Yamashita 2001, Journal of Biological Chemistry 276: 26745-26752, and WO 00/18889.

[0008] Higher plants comprise PUFA, such as linoleic acid and linolenic acid. However, the LCPUFA ARA, EPA and DHA are not present in the seed oils of higher plants or only in traces (Ucciani: Nouveau Dictionnaire des Huiles Végétales. Technique & Documentation-Lavoisier, 1995. ISBN: 2-7430-0009-0). It is nevertheless highly desirable to produce LCPUFA in higher plants, preferably in oil seeds such as oilseed rape, linseed, sunflower and soybean, since large amounts of high-quality LCPUFA for the various aforementioned applications may be obtained thereby at low costs.

[0009] WO 2009/085169 describes a sequences encoding an acyltransferase in SEO.ID.NO.54. WO2009/143398 describes a sequence encoding an lysophosphatidic acid acyltransferase protein (SEO.ID.NO. 159 and example 24) and further a sequence encoding a protein with acyltransferase activity (cf. example 24 and SEO.ID.No. 156). WO2007/106905 describes a sequence which encodes a diacylglycerolacyltransferase (cf. claim 34 and SEO.ID.NO. 119) and also a sequence which encodes an acyltransferase (cf. example 11 and SEO.ID.NO. 101). WO2009/143401 describes a sequence which encodes a polypeptide having diacylglycerol acyltransferase activity (cf. example 39 and SEO.ID.NO. 361).

[0010] However, one drawback of using transgenic plants expressing various of the aforementioned desaturases and elongases involved in the synthesis of PUFA and LCPUFA is that the latter are not efficiently incorporated into triacylglycerides, but rather into membranes. Furthermore, efficient processing of a given acyl molecule-substrate, e.g. linoleic acid, by a plurality of desaturation and elongation steps towards the desired LCPUFA, e.g. ARA, EPA and/or DHA, is hindered by the requirement to transfer the acyl molecule and its derivatives generated by the elongation and desaturation reactions back and forth between membrane lipids and acyl-CoA. For this reason, intermediates towards desired LCPUFA are incorporated into oil before the synthesis of the desired LCPUFA is complete. These two problems are undesired for the following reasons: First, the main lipid fraction in oil seeds are triacylglycerides. This is why, for economical reasons, it is necessary to concentrate LCPUFA in triacylglycerides. Second, LCPUFA which are incorporated into membranes can modify the physical characteristics of the membranes and thus have harmful effects on the integrity and transport characteristics of the membranes and on the stress tolerance of plants. Third, for efficient LCPUFA synthesis, it is desirable to increase the flux of intermediate-LCPUFA between the two sites of biosynthesis -that are membrane lipids and acyl-CoA - and/or decrease the flux of intermediate-PUFA-LCPUFA into oil. Transgenic plants which comprise and express genes coding for enzymes of LCPUFA biosynthesis and produce LCPUFA have been described, e.g., in DE 102 19 203 or WO2004/087902. However, these plants produce LCPUFA in amounts which require further optimization for processing the oils present in said plants. Moreover, it was proposed that delta 6 desaturated fatty acids may be shifted into the acyl-CoA pool for increasing efficiency of fatty acid elongation in plants (Singh 2005, Curr. Opin. Plant Biol., 8: 197-203). Another publication demonstrated in Arabidopsis, that the additional expression of RcDGAT2 from Ricinus communis increase the storage of hydroxyfatty acids produced by a Ricinus communis fatty acid hydroxylase 12 (FAH12) from 17% to 30% in the seed oil.

EP 2 585 603 B1

[0011] Accordingly, means for increasing the content of PUFA or LCPUFA, such as EPA and DHA, in triglycerides in, e.g., plant seed oils, are still highly desirable.

[0012] Thus, the present invention relates to a polynucleotide comprising a nucleic acid sequence selected from the group consisting of:

- a) a nucleic acid sequence consisting of the nucleotide sequence as shown in any one of SEQ ID NOs: 52, 7, 46 and 49;
- b) a nucleic acid sequence encoding a polypeptide having an amino acid sequence as shown in any one of SEQ ID NOs: 53, 8, 47 and 50;
- c) a nucleic acid sequence being at least 60% identical to the nucleic acid sequence as shown in any one of SEQ ID NOs: 52, 7, 46 and 49 or encoding a polypeptide having an amino acid sequence at least 60% identical to in any one of SEQ ID NOs: 53, 8, 47 and 50,

and wherein said nucleic acid sequence of (a) to (c) encodes a polypeptide having acyltransferase activity.

[0013] Matsuda et al. (Journal of Lipid Research, 2012, p1210ff, analysed the delta-12 fatty acid desaturase function of *T. aureum* and found that two distinct pathways are active in *T. aureum* for the synthesis of PUFAs. Zhang et al (Journal of Experimental Botany, 2013, Vol. 64, No. 11, pp 3189-3200) found that that the diacylglycerol acyltransferase 2 from *T. aureum*, has a broad substrate specificity and increases the oleic acid content in *A. thaliana* expressing said gene.

[0014] Herein also described is a polynucleotide comprising a nucleic acid sequence elected from the group consisting of:

- a) a nucleic acid sequence having a nucleotide sequence as shown in any one of SEQ ID NOs: 1, 4, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, and 55;
- b) a nucleic acid sequence encoding a polypeptide having an amino acid sequence as shown in any one of SEQ ID NOs: 2, 5, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, and 56;
- c) a nucleic acid sequence being at least 40% identical to the nucleic acid sequence of a) or b), wherein said nucleic acid sequence encodes a polypeptide having acyltransferase activity;
- d) a nucleic acid sequence encoding a polypeptide having acyltransferase activity and having an amino acid sequence which is at least 45% identical to the amino acid sequence of b); and
- e) a nucleic acid sequence which is capable of hybridizing under one of the following sets of conditions to any one of a) to d), wherein said nucleic acid sequence encodes a polypeptide having acyltransferase activity:
 - f) hybridization in 50 mM Tris, pH 7.6, 6xSSC, 5xDenhardt's, 1.0% sodium dodecyl sulfate (SDS) 100µg denaturated calf thymus DNA at 34°C overnight and wash twice with 2xSSC, 0.5%SDS at room temperature for 15 min each, repeat twice with 0.2xSSC, 0,5% SDS at room temperature for 15 min each and then repeat twice with 0.2 SSC, 0.5% SDS at 50°C for 15 min;
 - g) hybridization in 6xSSPE (Sodium chloride Sodium Phosphate-EDTA), 5xDenhardt's solution, 0.5% SDS 100µg denaturated calf thymus DNA at 34°C overnight and wash twice with 2xSSC, 0.5%SDS at room temperature for 15 min each, repeat twice with 0.2xSSC, 0,5% SDS at room temperature for 15 min each and then repeat twice with 0.2 SSC, 0.5% SDS at 50°C for 15 min;
 - h) hybridization in 20-30% formamide, 5xSSPE, 5xDenhardt's solution, 1% SDS 100µg denaturated salmon sperm DNA at 34°C overnight and wash twice with 2xSSPE, 0.2%SDS at 42°C for 15 min each, repeat twice with 2xSSPE, 0.2%SDS at 55°C for 30 min each and repeat twice with 0.2 SSC, 0.5% SDS at 50°C for 15 min;
 - i) hybridization in 7% SDS, 0.5 M NaPO₄, 1 mM EDTA at 50°C overnight and wash in 2 X SSC, 0.1% SDS at 50°C or 65°C;
 - j) hybridization in 7% SDS, 0.5 M NaPO₄, 1 mM EDTA at 50°C overnight and wash in 1 X SSC, 0.1% SDS at 50°C or 65°C; or
 - k) hybridization in 7% SDS, 0.5 M NaPO₄, 1 mM EDTA at 50°C overnight and wash in 0,1 X SSC, 0.1% SDS at 50°C or 65°C

[0015] 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 acyltransferase activity.

[0016] Preferably, the polypeptide encoded by the polynucleotide of the present invention having acyltransferase activity upon expression in a plant shall be capable of increasing the amount of PUFA and, in particular, LCPUFA esterified to triglycerides in, e.g., seed oils or the entire plant or parts thereof. Such an increase is, preferably, statistically significant when compared to a LCPUFA producing transgenic control plant which expresses the minimal set of desaturases and elongases required for LCPUFA synthesis but does not express the polynucleotide of the present invention. Such a transgenic plant may, preferably, express desaturases and elongases comprised by the vector LJB765 listed in table 11 of example 5 in WO2009/016202 or a similar set of desaturases and elongases required for DHA synthesis. Whether an increase is significant can be determined by statistical tests well known in the art including, e.g., Student's t-test.

More preferably, the increase is an increase of the amount of triglycerides containing LCPUFA of at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45% or at least 50% compared to the said control. Preferably, the LCPUFA referred to before is a polyunsaturated fatty acid having a C-20, C-22 or C24 fatty acid body, more preferably, EPA or DHA, most preferably, DHA. Suitable assays for measuring the

activities mentioned before are described in the accompanying Examples.

[0017] The term "acyltransferase activity" or "acyltransferase" as used herein encompasses all enzymatic activities and enzymes which are capable of transferring or are involved in the transfer of PUFA and, in particular; LCPUFA from the acyl-CoA pool or the membrane phospholipid to the triglycerides, from the acyl-CoA pool to membrane lipids and from membrane lipids to the acyl-CoA pool by a transesterification process. It will be understood that this acyltransferase activity will result in an increase of the LCPUFA esterified to triglycerides in, e.g., seed oils. In particular, it is envisaged that these acyltransferases are capable of producing triglycerides having esterified EPA or even DHA, or that these acyltransferases are capable of enhancing synthesis of desired PUFA by increasing the flux for specific intermediates of the desired PUFA between the acyl-CoA pool (the site of elongation) and membrane lipids (the predominant site of desaturation). Specifically, acyltransferase activity as used herein relates to lysophospholipid acyltransferase (LPLAT) activity, preferably, lysophosphatidylcholine acyltransferase (LPCAT) or Lysophosphatidylethanolamine acyltransferase (LPEAT) activity, lysophosphatidic acid acyltransferase (LPAAT) activity, glycerol-3-phosphate acyltransferase (GPAT) activity or diacylglycerol acyltransferase (DGAT), and, more preferably, to LPLAT, LPAAT, DGAT or GPAT activity.

[0018] More preferably, polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 1, 4, and 7, encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 2, 5, and 8 or variants thereof, preferably, exhibit LPLAT activity. Polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 10, and 13, encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 11, and 14 or variants thereof, preferably, exhibit LPAAT activity. Polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, 52, and 55, encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, and 56 or variants thereof, preferably, exhibit DGAT activity. A polynucleotide having a nucleic acid sequence as shown in SEQ ID NO: 55, encoding a polypeptide having amino acid sequences as shown in SEQ ID NO: 56 or variants thereof, preferably, exhibit GPAT activity.

[0019] A polynucleotide encoding a polypeptide having a acyltransferase activity as specified above has been obtained in accordance with the present invention, preferably, from *Nannochloropsis oculata* and/or *Thraustochytrium aureum*. However, orthologs, paralogs or other homologs may be identified from other species.

[0020] Thus, the term "polynucleotide" as used in accordance with the present invention further encompasses variants of the aforementioned specific polynucleotides representing orthologs, paralogs or other homologs of the polynucleotide of the present invention. Moreover, variants of the polynucleotide of the present invention also include artificially generated muteins. Said muteins include, e.g., enzymes which are generated by mutagenesis techniques and which exhibit improved or altered substrate specificity, or codon optimized polynucleotides.

[0021] The polynucleotide variants, preferably, comprise a nucleic acid sequence characterized in that the sequence can be derived from the aforementioned specific nucleic acid sequences shown in any one of SEQ ID NOs: 52, 7, 46 and 49 or by a polynucleotide encoding a polypeptide having an amino acid sequence as shown in any one of SEQ ID NOs: 53, 8, 47 and 50 by at least one nucleotide substitution, addition and/or deletion, whereby the variant nucleic acid sequence shall still encode a polypeptide having a acyltransferase activity as specified above.

[0022] Other polynucleotide variants, described herein, can preferably, comprise a nucleic acid sequence characterized in that the sequence can be derived from the aforementioned specific nucleic acid sequences shown in any one of SEQ ID NOs: 1, 4, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, and 55 or by a polynucleotide encoding a polypeptide having an amino acid sequence as shown in any one of SEQ ID NOs: 2, 5, , 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, and 56 by at least one nucleotide substitution, addition and/or deletion, whereby the variant nucleic acid sequence shall still encode a polypeptide having a acyltransferase activity as specified above.

[0023] 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 artisan 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 $6 \times$ sodium chloride/sodium citrate (= SSC) at approximately 45°C, followed by one or more wash steps in $0.2 \times$ SSC, 0.1% SDS at 50 to 65°C. The skilled artisan 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 $6 \times$ 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, $0.1 \times$ SSC and 20°C to 45°C, preferably between 30°C and 45°C and more preferably between 45°C and 65°C. The hybridization conditions for

DNA:RNA hybrids are, more preferably, $0.1 \times$ SSC and 30°C to 55°C , most preferably between 45°C and 65°C . The abovementioned hybridization temperatures are determined for example for a nucleic acid with approximately 100 bp (= base pairs) in length and a G + C content of 50% in the absence of formamide. The skilled artisan 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.

[0024] In detail variants of polynucleotides still encode a polypeptide having a acyltransferase activity as specified above comprising a nucleic acid sequence which is capable of hybridizing preferably under conditions equivalent to hybridization in 50 mM Tris, pH 7.6, 6xSSC, 5xDenhardt's, 1.0% sodium dodecyl sulfat (SDS) 100 μg denaturated calf thymus DNA at 34°C overnight, followed by washing twice with 2xSSC, 0.5%SDS at room temperature for 15 min each, then wash twice with 0.2xSSC, 0.5% SDS at room temperature for 15 min each and then wash twice with 0.2 SSC, 0.5% SDS at 50°C for 15 min each to a nucleic acid described by any one of SEQ ID NOs: 52, 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, and 55 or the complement thereof.

[0025] More preferably, said variants of polynucleotides comprising a nucleic acid sequence which is capable of hybridizing under conditions equivalent to hybridization in 6xSSPE (Sodium chloride Sodium Phosphate-EDTA), 5xDenhardt's solution, 0.5% sodium dodecyl sulfat (SDS) 100 μg denaturated calf thymus DNA at 34°C overnight, followed by washing twice with 2xSSC, 0.5%SDS at room temperature for 15 min each, then wash twice with 0.2xSSC, 0.5% SDS at room temperature for 15 min each and then wash twice with 0.2 SSC, 0.5% SDS at 50°C for 15 min each to a nucleic acid described by any one of SEQ ID NOs: 1, 4, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, and 55 or the complement thereof.

[0026] Most preferably, said variants of polynucleotides comprising a nucleic acid sequence which is capable of hybridizing under conditions equivalent to hybridization in 20-30% formamide, 5xSSPE (Sodium chloride Sodium Phosphate-EDTA), 5xDenhardt's solution, 1% sodium dodecyl sulfat (SDS) 100 μg denaturated salmon sperm DNA at 34°C overnight, followed by washing twice with 2xSSPE, 0.2%SDS at 42°C for 15 min each, then wash twice with 2xSSPE, 0.2%SDS at 55°C for 30 min each and then wash twice with 0.2 SSC, 0.5% SDS at 50°C for 15 min each to a nucleic acid described by any one of SEQ ID NOs: 1, 4, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, and 55 or the complement thereof.

[0027] In another preferred embodiment aforementioned variants of polynucleotides still encode a polypeptide having a acyltransferase activity as specified above comprising a nucleic acid sequence which is capable of hybridizing under conditions equivalent to hybridization in 7% sodium dodecyl sulfat (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C overnight with washing in 2 X SSC, 0.1% SDS at 50°C or 65°C , preferably 65°C to a nucleic acid described by any one of SEQ ID NOs: 1, 4, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, and 55 or the complement thereof. In still another preferred embodiment, said variants of polynucleotides comprising a nucleic acid sequence which is capable of hybridizing under conditions equivalent to hybridization in 7% sodium dodecyl sulfat (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C overnight with washing in 1 X SSC, 0.1% SDS at 50°C or 65°C , preferably 65°C to a nucleotide sequence described by any one of SEQ ID NOs: 1, 4, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, and 55 or or the complement thereof, most preferably, said variants of polynucleotides comprising a nucleic acid sequence which is capable of hybridizing under conditions equivalent to hybridization in 7% sodium dodecyl sulfat (SDS), 0.5 M Na-PO₄, 1 mM EDTA at 50°C overnight with washing in 0,1 X SSC, 0.1% SDS at 50°C or 65°C , preferably 65°C to a nucleic acid sequence described by any one of SEQ ID NOs: 1, 4, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, and 55 or the complement thereof.

[0028] The term "hybridization" as used herein includes "any process by which a strand of nucleic acid molecule joins with a complementary strand through base pairing." (J. Coombs (1994) Dictionary of Biotechnology, Stockton Press, New York). Hybridization and the strength of hybridization (i.e., the strength of the association between the nucleic acid molecules) is impacted by such factors as the degree of complementarity between the nucleic acid molecules, stringency of the conditions involved, the T_m of the formed hybrid, and the G:C ratio within the nucleic acid molecules. As used herein, the term "T_m" is used in reference to the "melting temperature." The melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. The equation for calculating the T_m of nucleic acid molecules is well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation: $T_m = 81.5 + 0.41 (\% \text{ G+C})$, when a nucleic acid molecule is in aqueous solution at 1 M NaCl [see e.g., Anderson and Young, Quantitative Filter Hybridization, in Nucleic Acid Hybridization (1985)]. Other references include more sophisticated computations, which take structural as well as sequence characteristics into account for the calculation of T_m. Stringent conditions, are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6.

[0029] A "complement" of a nucleic acid sequence as used herein refers to a nucleotide sequence whose nucleic acid molecules show total complementarity to the nucleic acid molecules of the nucleic acid sequence.

[0030] The term "Complementary" or "complementarity" refers to two nucleotide sequences which comprise antiparallel nucleotide sequences capable of pairing with one another (by the base-pairing rules) upon formation of hydrogen bonds

between the complementary base residues in the antiparallel nucleotide sequences. For example, the sequence 5'-AGT-3' is complementary to the sequence 5'-ACT-3'. Complementarity can be "partial" or "total." "Partial" complementarity is where one or more nucleic acid bases are not matched according to the base pairing rules. "Total" or "complete" complementarity between nucleic acid molecules is where each and every nucleic acid base is matched with another base under the base pairing rules. The degree of complementarity between nucleic acid molecule strands has significant effects on the efficiency and strength of hybridization between nucleic acid molecule strands.

[0031] Alternatively, polynucleotide variants are obtainable by PCR-based techniques such as mixed oligonucleotide primer-based amplification of DNA, i.e. using degenerated primers against conserved domains of the polypeptides of the present invention. Conserved domains of the polypeptide of the present invention may be identified by a sequence comparison of the nucleic acid sequences of the polynucleotides or the amino acid sequences of the polypeptides of the present invention. Oligonucleotides suitable as PCR primers as well as suitable PCR conditions are described in the accompanying Examples. As a template, DNA or cDNA from bacteria, fungi, plants or animals may be used.

[0032] Further, variants include polynucleotides comprising nucleic acid sequences which are at least up to at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the nucleic acid sequences shown in any one of SEQ ID NOs: 52, 7, 46 and 49, preferably, encoding polypeptides retaining a acyltransferase activity as specified above.

[0033] Further, variants of the nucleic acids described herein can include polynucleotides comprising nucleic acid sequences which are at least up to 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the nucleic acid sequences shown in any one of SEQ ID NOs: 1, 4, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, and 55, preferably, encoding polypeptides retaining a acyltransferase activity as specified above.

[0034] Moreover, also encompassed are polynucleotides (derivatives) which comprise nucleic acid sequences encoding a polypeptide having an amino acid sequences which are at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the amino acid sequences shown in any one of SEQ ID NOs: 53, 8, 47 and 50, wherein the polypeptide, preferably, retains acyltransferase activity as specified above.

[0035] Moreover, herein described are also polynucleotides (derivatives) which comprise nucleic acid sequences encoding a polypeptide having an amino acid sequences which are at least up to 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the amino acid sequences shown in any one of SEQ ID NOs: 2, 5, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, and 56, wherein the polypeptide, preferably, retains acyltransferase activity as specified above.

[0036] 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 artisan for comparing different sequences. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch algorithm (Needleman 1970, J. Mol. Biol. (48):444-453) which has been incorporated into the needle program in the EMBOSS software package (EMBOSS: The European Molecular Biology Open Software Suite, Rice,P., Longden,I., and Bleasby,A, Trends in Genetics 16(6), 276-277, 2000), using either a BLOSUM 45 or PAM250 scoring matrix for distantly related proteins, or either a BLOSUM 62 or PAM160 scoring matrix for closer related proteins, and a gap opening penalty of 16, 14, 12, 10, 8, 6, or 4 and a gap extension penalty of 0.5, 1, 2, 3, 4, 5, or 6. Guides for local installation of the EMBOSS package as well as links to WEB-Services can be found at <http://emboss.sourceforge.net>. A preferred, non-limiting example of parameters to be used for aligning two amino acid sequences using the needle program are the default parameters, including the EBLOSUM62 scoring matrix, a gap opening penalty of 10 and a gap extension penalty of 0.5. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the needle program in the EMBOSS software package (EMBOSS: The European Molecular Biology Open Software Suite, Rice,P., Longden,I., and Bleasby,A, Trends in Genetics 16(6), 276-277, 2000), using the EDNAFULL scoring matrix and a gap opening penalty of 16, 14, 12, 10, 8, 6, or 4 and a gap extension penalty of 0.5,1, 2, 3, 4, 5, or 6. A preferred, non-limiting example of parameters to be used in conjunction for aligning two amino acid sequences using the needle program are the default parameters, including the EDNAFULL scoring matrix, a gap opening penalty of 10 and a gap extension penalty of 0.5. The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the BLAST series of programs (version 2.2) of Altschul *et al.* (Altschul 1990, J. Mol. Biol. 215:403-10). BLAST using acyltransferase nucleic acid sequences of the invention as query sequence can be performed with the BLASTn, BLASTx or the tBLASTx program using default parameters to obtain either nucleotide sequences (BLASTn, tBLASTx) or amino acid sequences (BLASTx) homologous to acyltransferase sequences of the invention. BLAST using acyltransferase protein sequences of the invention as query

sequence can be performed with the BLASTp or the tBLASTn program using default parameters to obtain either amino acid sequences (BLASTp) or nucleic acid sequences (tBLASTn) homologous to acyltransferase sequences of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST using default parameters can be utilized as described in Altschul *et al.* (Altschul 1997, Nucleic Acids Res. 25(17):3389-3402).

Table 1: Relation of sequence types of query and hit sequences for various BLAST programs

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

[0037] 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 acyltransferase 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.

[0038] The variant polynucleotides or fragments referred to above, preferably, encode polypeptides retaining acyltransferase activity to a significant extent, preferably, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the acyltransferase activity exhibited by any of the polypeptide shown in any one of SEQ ID NOs: 53, 8, 47 and 50 or derivative of any of these polypeptides. The activity may be tested as described in the accompanying examples.

[0039] Also described variant polynucleotides or fragments referred to above, preferably, encode polypeptides retaining acyltransferase activity to a significant extent, preferably, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the acyltransferase activity exhibited by any of the polypeptide shown in any one of SEQ ID NOs: 2, 5, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 50, and 56 or derivative of any of these polypeptides. The activity may be tested as described in the accompanying examples.

[0040] The polynucleotides of the present invention either essentially consist of the aforementioned nucleic acid sequences or comprise the aforementioned nucleic acid sequences. Thus, they may contain further nucleic acid sequences as well. Preferably, the polynucleotide of the present invention may comprise in addition to an open reading frame further untranslated sequence at the 3' and at the 5' terminus of the coding gene region: at least 500, preferably 200, more preferably 100 nucleotides of the sequence upstream of the 5' terminus of the coding region and at least 100, preferably 50, more preferably 20 nucleotides of the sequence downstream of the 3' terminus of the coding gene region. Furthermore, the polynucleotides of the present invention may encode fusion proteins wherein one partner of the fusion protein is a polypeptide being encoded by a nucleic acid sequence recited above. Such fusion proteins may comprise as additional part other enzymes of the fatty acid or PUFA biosynthesis pathways, polypeptides for monitoring expression (e.g., green, yellow, blue or red fluorescent proteins, alkaline phosphatase and the like) or so called "tags" which may serve as a detectable marker or as an auxiliary measure for purification purposes. Tags for the different purposes are well known in the art and comprise FLAG-tags, 6-histidine-tags, MYC-tags and the like.

[0041] The polynucleotide of the present invention shall be provided, preferably, either as an isolated polynucleotide (i.e. purified or at least isolated from its natural context such as its natural gene locus) or in genetically modified or exogenously (i.e. artificially) manipulated form. An isolated polynucleotide can, for example, comprise less than approximately 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in the genomic DNA of the cell from which the nucleic acid is derived. The polynucleotide, preferably, is provided in the form of double or single stranded molecule. It will be understood that the present invention by referring to any of the aforementioned polynucleotides of the invention also refers to complementary or reverse complementary strands of the specific sequences or variants thereof referred to before. The polynucleotide encompasses DNA, including cDNA and genomic DNA, or RNA polynucleotides.

[0042] However, the present invention also pertains to polynucleotide variants which are derived from the polynucleotides of the present invention and are capable of interfering with the transcription or translation of the polynucleotides of the present invention. Such variant polynucleotides include anti-sense nucleic acids, ribozymes, siRNA molecules, morpholino nucleic acids (phosphorodiamidate morpholino oligos), triple-helix forming oligonucleotides, inhibitory oligonucleotides, or micro RNA molecules all of which shall specifically recognize the polynucleotide of the invention due to

the presence of complementary or substantially complementary sequences. These techniques are well known to the skilled artisan. Suitable variant polynucleotides of the aforementioned kind can be readily designed based on the structure of the polynucleotides of this invention.

5 [0043] 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.

10 [0044] Advantageously, it has been found in accordance with the present invention that the polynucleotides encoding the above mentioned polypeptides having acyltransferase activity and, in particular, LPLAT, LPAAT, DGAT and/or GPAT activity, can be used for the manufacture of PUFA and, in particular, LCPUFA when expressed in a transgenic host organism or cell. Specifically, the aforementioned acyltransferase activities will allow for an increase of LCPUFA esterified to triglycerides in seed oils by shifting the said LCPUFA from the acyl-CoA pool (by polypeptides having LPAAT, DGAT or GPAT activity as specified above) and/or from the acyl-CoA pool/phospholipid pool to the phospholipid pool/acyl-CoA pool (by polypeptides having LPLAT as specified above) via transesterification. Surprisingly, it was found that the acyltransferases encoded by the polynucleotides of the present invention are also capable of efficiently shifting rather long and highly unsaturated LCPUFA towards the triglyceride pool or between the phospholipid pool and the acyl-CoA pool, in particular, even the long chain intermediates. More surprisingly even, DHA which is known to be incorporated in triglycerides only in very low amounts, if at all, can be efficiently transesterified to triglycerides by the acyltransferases of the invention.

15 [0045] In particular the LPLAT can efficiently catalyse the transesterification of 18:2n-6 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterification of 18:2n-6 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterification of 18:2n-6 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), the transesterification of 18:3n-6 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterification of 18:3n-6 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterification of 18:3n-6 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), the transesterification of 18:3n-3 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterification of 18:3n-3 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterification of 18:3n-3 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), transesterification of 18:4n-3 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterification of 18:4n-3 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterification of 18:4n-3 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), the transesterification of 20:3n-6 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterification of 20:3n-6 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterification of 20:3n-6 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), the transesterification of 20:4n-3 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterification of 20:4n-3 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterification of 20:4n-3 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), the transesterification of 20:4n-6 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterification of 20:4n-6 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterification of 20:4n-6 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), the transesterification of 20:5n-3 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterification of 20:5n-3 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterification of 20:5n-3 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), the transesterification of 22:5n-3 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterification of 22:5n-3 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE), the transesterification of 22:5n-3 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS), the transesterification of 22:6n-3 from the sn2 position of phosphatidylcholine (PC) to CoA and/or from CoA to lysophosphatidylcholine (LPC), the transesterification of 22:6n-3 from the sn2 position of phosphatidylethanolamine (PE) to CoA and/or from CoA to lysophosphatidylethanolamine (LPE) and/or the transesterification of 22:6n-3 from the sn2 position of phosphatidylserine (PS) to CoA and/or from CoA to lysophosphatidylserine (LPS).

55 [0046] Preferably the LPAAT can efficiently catalyse the transesterification of 18:2n-6 from CoA to the sn2 position of lysophosphatidic acid (LPA), the transesterification of 18:3n-6 from CoA to the sn2 position of lysophosphatidic acid

(LPA), the transesterification of 18:3n-3 from CoA to the *sn2* position of lysophosphatidic acid (LPA) and/or the transesterification of 18:4n-6 from CoA to the *sn2* position of lysophosphatidic acid (LPA).

[0047] More preferably the LPAAT can efficiently catalyse the transesterification of 20:3n-6 from CoA to the *sn2* position of lysophosphatidic acid (LPA), transesterification of 20:4n-3 from CoA to the *sn2* position of lysophosphatidic acid (LPA) and/or the transesterification of 22:5n-3 from CoA to the *sn2* position of lysophosphatidic acid (LPA).

[0048] Most preferably the LPAAT can efficiently catalyse the transesterification of 20:4n-6 from CoA to the *sn2* position of lysophosphatidic acid (LPA), the transesterification of 20:5n-3 from CoA to the *sn2* position of lysophosphatidic acid (LPA) and/or the transesterification of 22:6n-3 from CoA to the *sn2* position of lysophosphatidic acid (LPA).

[0049] Preferably the GPAT can efficiently catalyse the transesterification of 18:2n-6 from CoA to the *sn1* position of glycerole-3-phosphate (G3P), the transesterification of 18:3n-6 from CoA to the *sn1* position of glycerole-3-phosphate (G3P), the transesterification of 18:3n-3 from CoA to the *sn1* position of glycerole-3-phosphate (G3P) and/or the transesterification of 18:4n-6 from CoA to the *sn1* position of glycerole-3-phosphate (G3P).

[0050] More preferably the GPAT can efficiently catalyse the transesterification of 20:3n-6 from CoA to the *sn1* position of glycerole-3-phosphate (G3P), the transesterification of 20:4n-3 from CoA to the *sn1* position of glycerole-3-phosphate (G3P) and/or the transesterification of 22:5n-3 from CoA to the *sn1* position of glycerole-3-phosphate (G3P).

[0051] Most preferably the GPAT can efficiently catalyse the transesterification of 20:4n-6 from CoA to the *sn1* position of glycerole-3-phosphate (G3P), the transesterification of 20:5n-3 from CoA to the *sn1* position of glycerole-3-phosphate (G3P) and/or the transesterification of 22:6n-3 from CoA to the *sn1* position of glycerole-3-phosphate (G3P).

[0052] Preferably the DGAT can efficiently catalyse the transesterification of 18:2n-6 from CoA to the *sn3* position of Diacylglycerol (DAG), transesterification of 18:3n-6 from CoA to the *sn3* position of Diacylglycerol (DAG), the transesterification of 18:3n-3 from CoA to the *sn3* position of Diacylglycerol (DAG) and/or the transesterification of 18:4n-6 from CoA to the *sn3* position of Diacylglycerol (DAG).

[0053] More preferably the DGAT can efficiently catalyse the transesterification of 20:3n-6 from CoA to the *sn3* position of Diacylglycerol (DAG), the transesterification of 20:4n-3 from CoA to the *sn3* position of Diacylglycerol (DAG) and/or the transesterification of 22:5n-3 from CoA to the *sn3* position of Diacylglycerol (DAG).

[0054] Most preferably the DGAT can efficiently catalyse the transesterification of 20:4n-6 from CoA to the *sn3* position of Diacylglycerol (DAG), the transesterification of 20:5n-3 from CoA to the *sn3* position of Diacylglycerol (DAG) and/or the transesterification of 22:6n-3 from CoA to the *sn3* position of Diacylglycerol (DAG).

[0055] The activity of the LPLAT, LPAAT, GPAT or DGAT is useful for the specificity of a fatty acid. This fatty acid specificity is useful to generate an artificially ARA-specificity preferably. More preferably the activity of the LPLAT, LPAAT, GPAT or DGAT of the present invention is useful to generate an artificially EPA-specificity. Most preferably the activity of the LPLAT, LPAAT, GPAT or DGAT of the present invention is useful to generate an artificially DHA-specificity.

[0056] In a preferred embodiment of the polynucleotide of the present invention, said polynucleotide further comprises an expression control sequence operatively linked to the said nucleic acid sequence.

[0057] The term "expression control sequence" as used herein refers to a nucleic acid sequence which is capable of governing, i.e. initiating and controlling, transcription of a nucleic acid sequence of interest, in the present case the nucleic sequences recited above. Such a sequence usually comprises or consists of a promoter or a combination of a promoter and enhancer sequences. Expression of a polynucleotide comprises transcription of the nucleic acid molecule, preferably, into a translatable mRNA. Additional regulatory elements may include transcriptional as well as translational enhancers. The following promoters and expression control sequences may be, preferably, used in an expression vector according to the present invention. The *cos*, *tac*, *trp*, *tet*, *trp-tet*, *lpp*, *lac*, *lpp-lac*, *lacIq*, T7, T5, T3, *gal*, *trc*, *ara*, SP6, λ -PR or λ -PL promoters are, preferably, used in Gram-negative bacteria. For Gram-positive bacteria, promoters *amy* and *SPO2* may be used. From yeast or fungal promoters *ADC1*, *AOX1r*, *GAL1*, *MF α* , *AC*, *P-60*, *CYC1*, *GAPDH*, *TEF*, *rp28*, *ADH* are, preferably, used. For animal cell or organism expression, the promoters *CMV-*, *SV40-*, *RSV-promoter* (Rous sarcoma virus), *CMV-enhancer*, *SV40-enhancer* are preferably used. From plants the promoters *CaMV/35S* (Franck 1980, Cell 21: 285-294), *PRP1* (Ward 1993, Plant. Mol. Biol. 22), *SSU*, *OCS*, *lib4*, *usp*, *STLS1*, *B33*, *nos* or the ubiquitin or phaseolin promoter. Also preferred in this context are inducible promoters, such as the promoters described in EP 0 388 186 A1 (i.e. a benzylsulfonamide-inducible promoter), Gatz 1992, Plant J. 2:397-404 (i.e. a tetracyclin-inducible promoter), EP 0 335 528 A1 (i.e. an abscisic-acid-inducible promoter) or WO 93/21334 (i.e. an ethanol- or cyclohexanol-inducible promoter). Further suitable plant promoters are the promoter of cytosolic FBPase or the *ST-LSI* promoter from potato (Stockhaus 1989, EMBO J. 8, 2445), the phosphoribosyl-pyrophosphate amidotransferase promoter from Glycine max (Genbank accession No. U87999) or the node-specific promoter described in EP 0 249 676 A1. Particularly preferred are promoters which enable the expression in tissues which are involved in the biosynthesis of fatty acids. Also particularly preferred are seed-specific promoters such as the *USP* promoter in accordance with the practice, but also other promoters such as the *LeB4*, *DC3*, *phaseolin* or *napin* promoters. Further especially preferred promoters are seed-specific promoters which can be used for monocotyledonous or dicotyledonous plants and which are described in US 5,608,152 (*napin* promoter from oilseed rape), WO 98/45461 (*oleosin* promoter from Arabidopsis), US 5,504,200 (*phaseolin* promoter from Phaseolus vulgaris), WO 91/13980 (*Bce4* promoter from Brassica), by Baeumlein et al., Plant J., 2, 2, 1992:233-239

(LeB4 promoter from a legume), these promoters being suitable for dicots. The following promoters are suitable for monocots: lpt-2 or lpt-1 promoter from barley (WO 95/15389 and WO 95/23230), hordein promoter from barley and other promoters which are suitable and which are described in WO 99/16890. In principle, it is possible to use all natural promoters together with their regulatory sequences, such as those mentioned above, for the novel process. Likewise, it is possible and advantageous to use synthetic promoters, either additionally or alone, especially when they mediate a seed-specific expression, such as, for example, as described in WO 99/16890. In a particular embodiment, seed-specific promoters are utilized to enhance the production of the desired PUFA or LCPUFA.

[0058] The term "operatively linked" as used herein means that the expression control sequence and the nucleic acid of interest are linked so that the expression of the said nucleic acid of interest can be governed by the said expression control sequence, i.e. the expression control sequence shall be functionally linked to the said nucleic acid sequence to be expressed. Accordingly, the expression control sequence and, the nucleic acid sequence to be expressed may be physically linked to each other, e.g., by inserting the expression control sequence at the 5' end of the nucleic acid sequence to be expressed. Alternatively, the expression control sequence and the nucleic acid to be expressed may be merely in physical proximity so that the expression control sequence is capable of governing the expression of at least one nucleic acid sequence of interest. The expression control sequence and the nucleic acid to be expressed are, preferably, separated by not more than 500 bp, 300 bp, 100 bp, 80 bp, 60 bp, 40 bp, 20 bp, 10 bp or 5 bp.

[0059] In a further preferred embodiment of the polynucleotide of the present invention, said polynucleotide further comprises a terminator sequence operatively linked to the nucleic acid sequence.

[0060] The term "terminator" as used herein refers to a nucleic acid sequence which is capable of terminating transcription. These sequences will cause dissociation of the transcription machinery from the nucleic acid sequence to be transcribed. Preferably, the terminator shall be active in plants and, in particular, in plant seeds. Suitable terminators are known in the art and, preferably, include polyadenylation signals such as the SV40-poly-A site or the tk-poly-A site or one of the plant specific signals indicated in Loke et al. 2005, *Plant Physiol* 138, pp. 1457-1468, downstream of the nucleic acid sequence to be expressed.

[0061] The present invention also relates to a vector comprising the polynucleotide of the present invention.

[0062] The term "vector", preferably, encompasses phage, plasmid, viral vectors as well as artificial chromosomes, such as bacterial or yeast artificial chromosomes. Moreover, the term also relates to targeting constructs which allow for random or site-directed integration of the targeting construct into genomic DNA. Such target constructs, preferably, comprise DNA of sufficient length for either homologous or heterologous recombination as described in detail below. The vector encompassing the polynucleotide of the present invention, preferably, further comprises selectable markers for propagation and/or selection in a host. The vector may be incorporated into a host cell by various techniques well known in the art. If introduced into a host cell, the vector may reside in the cytoplasm or may be incorporated into the genome. In the latter case, it is to be understood that the vector may further comprise nucleic acid sequences which allow for homologous recombination or heterologous insertion. Vectors can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. The terms "transformation" and "transfection", conjugation and transduction, as used in the present context, are intended to comprise a multiplicity of prior-art processes for introducing foreign nucleic acid (for example DNA) into a host cell, including calcium phosphate, rubidium chloride or calcium chloride coprecipitation, 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.

[0063] 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. Jené et al., *Techniques for Gene Transfer*, in: *Transgenic Plants*, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press (1993), 128-143; Potrykus 1991, *Annu. Rev. Plant Physiol. Plant Molec. Biol.* 42, 205-225.

[0064] More preferably, the vector of the present invention is an expression vector. In such an expression vector, i.e. a vector which comprises the polynucleotide of the invention having the nucleic acid sequence operatively linked to an expression control sequence (also called "expression cassette") allowing expression in prokaryotic or eukaryotic cells or isolated fractions thereof. Suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (Invitrogen) or pSPORT1 (GIBCO BRL). Further examples of typical fusion expression vectors are pGEX (Pharmacia Biotech Inc; Smith 1988, *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ), where glutathione S-transferase (GST), maltose E-binding protein and protein A, respectively, are fused with the recombinant target protein. Examples of suitable inducible nonfusion *E. coli* expression vectors are, inter alia, pTrc (Amann 1988, *Gene* 69:301-315) and pET 11d (Studier 1990, *Methods in Enzymology* 185, 60-89). The target gene expression of the pTrc vector is based on the transcription from a hybrid trp-lac fusion promoter by host RNA polymerase. The target gene expression from the pET 11d vector is based on the transcription of a T7-gn10-lac fusion promoter, which is mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is provided by the host strains BL21 (DE3) or HMS174 (DE3) from a resident λ -prophage which harbors a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter. The skilled artisan 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, λ gt11 or pBdCl, in *Streptomyces* pIJ101, pIJ364, pIJ702 or pIJ361, in *Bacillus* pUB110, pC194 or pBD214, in *Corynebacterium* pSA77 or pAJ667. Examples of vectors for expression in the yeast *S. cerevisiae* comprise pYep Sec1 (Baldari 1987, *Embo J.* 6:229-234), pMFa (Kurjan 1982, *Cell* 30:933-943), pJRY88 (Schultz 1987, *Gene* 54:113-123) and pYES2 (Invitrogen Corporation, San Diego, CA). Vectors and processes for the construction of vectors which are suitable for use in other fungi, such as the filamentous fungi, comprise those which are described in detail in: van den Hondel, C.A.M.J.J., & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: *Applied Molecular Genetics of fungi*, J.F. Peberdy et al., Ed., pp. 1-28, Cambridge University Press: Cambridge, or in: *More Gene Manipulations in Fungi* (J.W. Bennett & L.L. Lasure, Ed., pp. 396-428: Academic Press: San Diego). Further suitable yeast vectors are, for example, pAG-1, YEp6, YEp13 or pEMBLye23. As an alternative, the polynucleotides of the present invention can be also expressed in insect cells using baculovirus expression vectors. Baculovirus vectors which are available for the expression of proteins in cultured insect cells (for example Sf9 cells) comprise the pAc series (Smith 1983, *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow 1989, *Virology* 170:31-39).

[0065] The polynucleotide of the present invention can be expressed in single-cell plant cells (such as algae), see Falciatore 1999, *Marine Biotechnology* 1 (3):239-251 and the references cited therein, and plant cells from higher plants (for example Spermatophytes, such as arable crops) by using plant expression vectors. Examples of plant expression vectors comprise those which are described in detail in: Becker 1992, *Plant Mol. Biol.* 20:1195-1197; Bevan 1984, *Nucl. Acids Res.* 12:8711-8721; *Vectors for Gene Transfer in Higher Plants*; in: *Transgenic Plants*, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, p. 15-38. A plant expression cassette, preferably, comprises regulatory sequences which are capable of controlling the gene expression in plant cells and which are functionally linked so that each sequence can fulfill its function, such as transcriptional termination, for example polyadenylation signals. Preferred polyadenylation signals are those which are derived from *Agrobacterium tumefaciens* T-DNA, such as the gene 3 of the Ti plasmid pTiACH5, which is known as octopine synthase (Gielen 1984, *EMBO J.* 3, 835) or functional equivalents of these, but all other terminators which are functionally active in plants are also suitable. Since plant gene expression is very often not limited to transcriptional levels, a plant expression cassette preferably comprises other functionally linked sequences such as translation enhancers, for example the overdrive sequence, which comprises the 5'-untranslated tobacco mosaic virus leader sequence, which increases the protein/RNA ratio (Gallie 1987, *Nucl. Acids Research* 15:8693-8711). As described above, plant gene expression must be functionally linked to a suitable promoter which performs the expression of the gene in a timely, cell-specific or tissue-specific manner. Promoters which can be used are constitutive promoters (Benfey 1989, *EMBO J.* 8:2195-2202) such as those which are derived from plant viruses such as 35S CAMV (Franck 1980, *Cell* 21:285-294), 19S CaMV (see US 5,352,605 and WO 84/02913) or plant promoters such as the promoter of the Rubisco small subunit, which is described in US 4,962,028. Other preferred sequences for the use in functional linkage in plant gene expression cassettes are targeting sequences which are required for targeting the gene product into its relevant cell compartment (for a review, see Kermode 1996, *Crit. Rev. Plant Sci.* 15, 4: 285-423 and references cited therein), for example into the vacuole, the nucleus, all types of plastids,

such as amyloplasts, chloroplasts, chromoplasts, the extracellular space, the mitochondria, the endoplasmic reticulum, oil bodies, peroxisomes and other compartments of plant cells. As described above, plant gene expression can also be facilitated via a chemically inducible promoter (for a review, see Gatz 1997, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 48:89-108). Chemically inducible promoters are particularly suitable if it is desired that genes are expressed in a time-specific manner. Examples of such promoters are a salicylic-acid-inducible promoter (WO 95/19443), a tetracyclin-inducible promoter (Gatz 1992, *Plant J.* 2, 397-404) and an ethanol-inducible promoter. Promoters which respond to biotic or abiotic stress conditions are also suitable promoters, for example the pathogen-induced PRP1-gene promoter (Ward 1993, *Plant Mol. Biol.* 22:361-366), the heat-inducible hsp80 promoter from tomato (US 5,187,267), the cold-inducible alpha-amylase promoter from potato (WO 96/12814) or the wound-inducible pinII promoter (EP 0 375 091 A). The promoters which are especially preferred are those which bring about the expression of genes in tissues and organs in which fatty acid, lipid and oil biosynthesis takes place, in seed cells such as the cells of endosperm and of the developing embryo. Suitable promoters are the napin gene promoter from oilseed rape (US 5,608,152), the USP promoter from *Vicia faba* (Baeumlein 1991, *Mol. Gen. Genet.* 225 (3):459-67), the oleosin promoter from *Arabidopsis* (WO 98/45461), the phaseolin promoter from *Phaseolus vulgaris* (US 5,504,200), the Bce4 promoter from *Brassica* (WO 91/13980) or the legumin B4 promoter (LeB4; Baeumlein 1992, *Plant Journal*, 2 (2):233-9), and promoters which bring about the seed-specific expression in monocotyledonous plants such as maize, barley, wheat, rye, rice and the like. Suitable promoters to be taken into consideration are the lpt2 or lpt1 gene promoter from barley (WO 95/15389 and WO 95/23230) or those which are described in WO 99/16890 (promoters from the barley hordein gene, the rice glutelin gene, the rice oryzin gene, the rice prolamin gene, the wheat gliadin gene, wheat glutelin gene, the maize zein gene, the oat glutelin gene, the sorghum kasirin gene, the rye secalin gene). Likewise, especially suitable are promoters which bring about the plastid-specific expression since plastids are the compartment in which the precursors and some end products of lipid biosynthesis are synthesized. Suitable promoters such as the viral RNA-polymerase promoter are described in WO 95/16783 and WO 97/06250, and the clpP promoter from *Arabidopsis*, described in WO 99/46394.

[0066] 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 artisan and are described, for example, in: *Cloning Vectors* (Ed., Pouwels, P.H., et al., Elsevier, Amsterdam-New York-Oxford, 1985, ISBN 0 444 904018). For further suitable expression systems for prokaryotic and eukaryotic cells see the chapters 16 and 17 of Sambrook, loc cit.

[0067] It follows from the above that, preferably, said vector is an expression vector. More preferably, the said polynucleotide of the present invention is under the control of a seed-specific promoter in the vector of the present invention. A preferred seed-specific promoter as meant herein is selected from the group consisting of Conlinin 1, Conlinin 2, napin, LuFad3, USP, LeB4, Arc, Fae, ACP, LuPXR, and SBP. For details, see, e.g., US 2003-0159174.

[0068] Moreover, the present invention relates to a host cell comprising the polynucleotide or the vector of the present invention.

[0069] Preferably, said host cell is a plant cell and, more preferably, a plant cell obtained from an oilseed crop. More preferably, said oilseed crop is selected from the group consisting of flax (*Linum* sp.), rapeseed (*Brassica* sp.), soybean (*Glycine* and *Soja* sp.), sunflower (*Helianthus* sp.), cotton (*Gossypium* sp.), corn (*Zea mays*), olive (*Olea* sp.), safflower (*Carthamus* sp.), cocoa (*Theobroma cacao*), peanut (*Arachis* sp.), hemp, camelina, crambe, oil palm, coconuts, groundnuts, sesame seed, castor bean, lesquerella, tallow tree, sheanuts, tungnuts, kapok fruit, poppy seed, jojoba seeds and perilla.

[0070] Also preferably, said host cell is a microorganism. More preferably, said microorganism is a bacterium, a fungus or algae. More preferably, it is selected from the group consisting of *Candida*, *Cryptococcus*, *Lipomyces*, *Rhodosporidium*, *Yarrowia* and *Schizochytrium*.

[0071] Moreover, a host cell according to the present invention may also be an animal cell. Preferably, said animal host cell is a host cell of a fish or a cell line obtained therefrom. More preferably, the fish host cell is from herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna.

[0072] It will be understood that if the host cell of the invention shall be applied for LCPUFA production, it shall be capable of carrying out desaturation and elongation steps on fatty acids. To produce the LCPUFA according to the invention, the C16- or C18- fatty acids must first be desaturated by the enzymatic activity of a desaturase and subsequently be elongated by at least two carbon atoms via an elongase. After one elongation cycle, this enzyme activity gives C18- or C20-fatty acids and after two or three elongation cycles C22- or C24-fatty acids. The activity of the desaturases and elongases used in the process according to the invention preferably leads to C18-, C20-, C22- and/or C24-fatty acids, advantageously with at least two double bonds in the fatty acid molecule, preferably with three, four or five double bonds, especially preferably to give C20- and/or C22-fatty acids with at least two double bonds in the fatty acid molecule, preferably with three, four or five double bonds in the molecule. After a first desaturation and the elongation have taken place, further desaturation steps such as, for example, one in the delta-5 position may take place. Products of the process according to the invention which are especially preferred are DGLA, ARA, EPA DPA and/or DHA, most preferably EPA and/or DHA. Desaturases and elongases which are required for this process may not always be present naturally in the host cell. Accordingly, the present invention, preferably, envisages a host cell which in addition to the polynucleotide of

the present invention comprises polynucleotides encoding such desaturases and/or elongases as required depending on the selected organism. Preferred desaturases and/or elongases which shall be present in the host cell are at least one enzyme selected from the group consisting of: Δ -4-desaturase, Δ -5-desaturase, Δ -5-elongase, Δ -6-desaturase, Δ 12-desaturase, Δ 15-desaturase, ω 3-desaturase and Δ -6-elongase. Especially preferred are the bifunctional d12d15-Desaturases d12d15Des(Ac) from *Acanthamoeba castellanii* (WO2007042510), d12d15Des(Cp) from *Claviceps purpurea* (WO2008006202) and d12d15Des(Lg)1 from *Lottia gigantea* (WO2009016202), the d12-Desaturases d12Des(Co) from *Calendula officinalis* (WO200185968), d12Des(Lb) from *Laccaria bicolor* (WO2009016202), d12Des(Mb) from *Monosiga brevicollis* (WO2009016202), d12Des(Mg) from *Mycosphaerella graminicola* (WO2009016202), d12Des(Nh) from *Nectria haematococca* (WO2009016202), d12Des(Ol) from *Ostreococcus lucimarinus* (WO2008040787), d12Des(Pb) from *Phycomyces blakesleeanus* (WO2009016202), d12Des(Ps) from *Phytophthora sojae* (WO2006100241) and d12Des(Tp) from *Thalassiosira pseudonana* (WO2006069710), the d15-Desaturases d15Des(Hr) from *Helobdella robusta* (WO2009016202), d15Des(Mc) from *Microcoleus chthonoplastes* (WO2009016202), d15Des(Mf) from *Mycosphaerella fijiensis* (WO2009016202), d15Des(Mg) from *Mycosphaerella graminicola* (WO2009016202) and d15Des(Nh)2 from *Nectria haematococca* (WO2009016202), the d4-Desaturases d4Des(Eg) from *Euglena gracilis* (WO2004090123), d4Des(Tc) from *Thraustochytrium* sp. (WO2002026946) and d4Des(Tp) from *Thalassiosira pseudonana* (WO2006069710), the d5-Desaturases d5Des(Ol)2 from *Ostreococcus lucimarinus* (WO2008040787), d5Des(Pp) from *Physcomitrella patens* (WO2004057001), d5Des(Pt) from *Phaeodactylum tricornutum* (WO2002057465), d5Des(Tc) from *Thraustochytrium* sp. (WO2002026946), d5Des(Tp) from *Thalassiosira pseudonana* (WO2006069710) and the d6-Desaturases d6Des(Cp) from *Ceratodon purpureus* (WO2000075341), d6Des(Ol) from *Ostreococcus lucimarinus* (WO2008040787), d6Des(Ot) from *Ostreococcus tauri* (WO2006069710), d6Des(Pf) from *Primula farinosa* (WO2003072784), d6Des(Pir_BO) from *Pythium irregulare* (WO2002026946), d6Des(Pir) from *Pythium irregulare* (WO2002026946), d6Des(Plu) from *Primula luteola* (WO2003072784), d6Des(Pp) from *Physcomitrella patens* (WO200102591), d6Des(Pt) from *Phaeodactylum tricornutum* (WO2002057465), d6Des(Pv) from *Primula vialii* (WO2003072784) and d6Des(Tp) from *Thalassiosira pseudonana* (WO2006069710), the d8-Desaturases d8Des(Ac) from *Acanthamoeba castellanii* (EP1790731), d8Des(Eg) from *Euglena gracilis* (WO200034439) and d8Des(Pm) from *Perkinsus marinus* (WO2007093776), the ω 3-Desaturases ω 3Des(Pi) from *Phytophthora infestans* (WO2005083053), ω 3Des(Pir) from *Pythium irregulare* (WO2008022963), ω 3Des(Pir)2 from *Pythium irregulare* (WO2008022963) and ω 3Des(Ps) from *Phytophthora sojae* (WO2006100241), the bifunctional d5d6-elongases d5d6Elo(Om)2 from *Oncorhynchus mykiss* (WO2005012316), d5d6Elo(Ta) from *Thraustochytrium aureum* (WO2005012316) and d5d6Elo(Tc) from *Thraustochytrium* sp. (WO2005012316), the d5-elongases d5Elo(At) from *Arabidopsis thaliana* (WO2005012316), d5Elo(At)2 from *Arabidopsis thaliana* (WO2005012316), d5Elo(Ci) from *Ciona intestinalis* (WO2005012316), d5Elo(Ol) from *Ostreococcus lucimarinus* (WO2008040787), d5Elo(Ot) from *Ostreococcus tauri* (WO2005012316), d5Elo(Tp) from *Thalassiosira pseudonana* (WO2005012316) and d5Elo(Xl) from *Xenopus laevis* (WO2005012316), the d6-elongases d6Elo(Ol) from *Ostreococcus lucimarinus* (WO2008040787), d6Elo(Ot) from *Ostreococcus tauri* (WO2005012316), d6Elo(Pi) from *Phytophthora infestans* (WO2003064638), d6Elo(Pir) from *Pythium irregulare* (WO2009016208), d6Elo(Pp) from *Physcomitrella patens* (WO2001059128), d6Elo(Ps) from *Phytophthora sojae* (WO2006100241), d6Elo(Ps)2 from *Phytophthora sojae* (WO2006100241), d6Elo(Ps)3 from *Phytophthora sojae* (WO2006100241), d6Elo(Pt) from *Phaeodactylum tricornutum* (WO2005012316), d6Elo(Tc) from *Thraustochytrium* sp. (WO2005012316) and d6Elo(Tp) from *Thalassiosira pseudonana* (WO2005012316), the d9-elongases d9Elo(Ig) from *Isochrysis galbana* (WO2002077213), d9Elo(Pm) from *Perkinsus marinus* (WO2007093776) and d9Elo(Ro) from *Rhizopus oryzae* (WO2009016208).

[0073] The present invention also relates to a cell, preferably a host cell as specified above or a cell of a non-human organism specified elsewhere herein, said cell comprising a polynucleotide which is obtained from the polynucleotide of the present invention by a point mutation, a truncation, an inversion, a deletion, an addition, a substitution and homologous recombination. How to carry out such modifications to a polynucleotide is well known to the skilled artisan and has been described elsewhere in this specification in detail.

[0074] The present invention furthermore relates to a method for the manufacture of a polypeptide encoded by a polynucleotide of any the present invention comprising

- a) cultivating the host cell of the invention under conditions which allow for the production of said polypeptide; and
- b) obtaining the polypeptide from the host cell of step a).

[0075] Suitable conditions which allow for expression of the polynucleotide of the invention comprised by the host cell depend on the host cell as well as the expression control sequence used for governing expression of the said polynucleotide. These conditions and how to select them are very well known to those skilled in the art. The expressed polypeptide may be obtained, for example, by all conventional purification techniques including affinity chromatography, size exclusion chromatography, high pressure liquid chromatography (HPLC) and precipitation techniques including antibody precipitation. It is to be understood that the method may - although preferred - not necessarily yield an essentially

pure preparation of the polypeptide. It is to be understood that depending on the host cell which is used for the aforementioned method, the polypeptides produced thereby may become posttranslationally modified or processed otherwise.

[0076] The present invention encompasses a polypeptide encoded by the polynucleotide of the present invention or which is obtainable by the aforementioned method.

[0077] The term "polypeptide" as used herein encompasses essentially purified polypeptides or polypeptide preparations comprising other proteins in addition. Further, the term also relates to the fusion proteins or polypeptide fragments being at least partially encoded by the polynucleotide of the present invention referred to above. Moreover, it includes chemically modified polypeptides. Such modifications may be artificial modifications or naturally occurring modifications such as phosphorylation, glycosylation, myristylation and the like (Review in Mann 2003, Nat. Biotechnol. 21, 255-261, review with focus on plants in Huber 2004, Curr. Opin. Plant Biol. 7, 318-322). Currently, more than 300 posttranslational modifications are known (see full ABFRC Delta mass list at <http://www.abrf.org/index.cfm/dm.home>). The polypeptide of the present invention shall exhibit the acyltransferase activities referred to above.

[0078] The present invention furthermore relates to an antibody or a fragment derived thereof as an antigen which specifically recognizes a polypeptide encoded by the nucleic acid sequences of the invention.

[0079] Antibodies against the polypeptides of the invention can be prepared by well known methods using a purified polypeptide according to the invention or a suitable fragment derived therefrom as an antigen. A fragment which is suitable as an antigen may be identified by antigenicity determining algorithms well known in the art. Such fragments may be obtained either from the polypeptide of the invention by proteolytic digestion or may be a synthetic peptide. Preferably, the antibody of the present invention is a monoclonal antibody, a polyclonal antibody, a single chain antibody, a chimerized antibody or a fragment of any of these antibodies, such as Fab, Fv or scFv fragments etc.. Also comprised as antibodies by the present invention are bispecific antibodies, synthetic antibodies or chemically modified derivatives of any of the aforementioned antibodies. The antibody of the present invention shall specifically bind (i.e. does significantly not cross react with other polypeptides or peptides) to the polypeptide of the invention. Specific binding can be tested by various well known techniques. Antibodies or fragments thereof can be obtained by using methods which are described, e.g., in Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1988. Monoclonal antibodies can be prepared by the techniques originally described in Köhler 1975, Nature 256, 495, and Galfre 1981, Meth. Enzymol. 73, 3, which comprise the fusion of mouse myeloma cells to spleen cells derived from immunized mammals. The antibodies can be used, for example, for the immunoprecipitation, immunolocalization or purification (e.g., by affinity chromatography) of the polypeptides of the invention as well as for the monitoring of the presence of said variant polypeptides, for example, in recombinant organisms, and for the identification of proteins or compounds interacting with the proteins according to the invention.

[0080] Moreover, the present invention contemplates a non-human transgenic organism comprising the polynucleotide or the vector of the present invention.

[0081] Preferably, the non-human transgenic organism is a microorganism, more preferably the non-human transgenic organism is a insect cell, bacterium or algae and most preferably the non-human transgenic organism is a plant, plant part, or plant seed. Preferred plants to be used for introducing the polynucleotide or the vector of the invention are plants which are capable of synthesizing fatty acids, such as all dicotyledonous or monocotyledonous plants, algae or mosses. It is to be understood that host cells derived from a plant may also be used for producing a plant according to the present invention. Preferred plants are selected from the group of the plant families Adoltheciaceae, Anacardiaceae, Asteraceae, Apiaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Caricaceae, Cannabaceae, Convolvulaceae, Chenopodiaceae, Cryptocodiaceae, 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: Adoltheciaceae 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 annuus [sunflower], Lactuca sativa, Lactuca crispera, 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, Arabidopsis, for example the genera and species Brassica napus, Brassica rapa ssp. [oilseed rape], Sinapis arvensis Brassica juncea, Brassica juncea var. juncea, Brassica juncea var. crispifolia, Brassica juncea var. foliosa, Brassica nigra, Brassica sinapioides, Melanosinapis communis [mustard], Brassica oleracea [fodder beet] or Arabidopsis thaliana, Bromeliaceae,

such as the genera *Anana*, *Bromelia* (pineapple), for example the genera and species *Anana comosus*, *Ananas ananas* or *Bromelia comosa* [pineapple], *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 *Ipomea batatas*, *Ipomea pandurata*, *Convolvulus batatas*, *Convolvulus tiliaceus*, *Ipomea fastigiata*, *Ipomea tiliacea*, *Ipomea 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 *Crypthecodinium 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 tricorutum*, *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 crispatisimum*, *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*, *Medicago*, *Glycine*, *Dolichos*, *Phaseolus*, *Soja*, for example the genera and species *Pisum sativum*, *Pisum arvense*, *Pisum humile* [pea], *Albizia berteriana*, *Albizia julibrissin*, *Albizia lebeck*, *Acacia berteriana*, *Acacia littoralis*, *Albizia berteriana*, *Albizia berteriana*, *Cathormion berteriana*, *Feuillea berteriana*, *Inga fragrans*, *Pithecellobium berterianum*, *Pithecellobium fragrans*, *Pithecolobium berterianum*, *Pseudalbizia berteriana*, *Acacia julibrissin*, *Acacia nemu*, *Albizia nemu*, *Feuillea julibrissin*, *Mimosa julibrissin*, *Mimosa speciosa*, *Sericandra julibrissin*, *Acacia lebeck*, *Acacia macrophylla*, *Albizia lebeck*, *Feuillea lebeck*, *Mimosa lebeck*, *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 *Aphanorrhagma*, *Entosthodon*, *Funaria*, *Physcomitrella*, *Physcomitrium*, for example the genera and species *Aphanorrhagma 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 mühlenbergii*, *Funaria orcuttii*, *Funaria plano-convexa*, *Funaria polaris*, *Funaria ravenelii*, *Funaria rubriseta*, *Funaria serrata*, *Funaria sonora*, *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 washing-toniense*, *Geraniaceae*, such as the genera *Pelargonium*, *Cocos*, *Oleum*, for example the genera and species *Cocos nucifera*, *Pelargonium grossularioides* or *Oleum cocois* [coconut], *Gramineae*, such as the genus *Saccharum*, for example the genus and species *Saccharum officinarum*, *Juglandaceae*, such as the genera *Juglans*, *Wallia*, for example the genera and species *Juglans regia*, *Juglans ailanthifolia*, *Juglans sieboldiana*, *Juglans cinerea*, *Wallia cinerea*, *Juglans bixbyi*, *Juglans californica*, *Juglans hindsii*, *Juglans intermedia*, *Juglans jamaicensis*, *Juglans major*, *Juglans microcarpa*, *Juglans nigra* or *Wallia nigra* [walnut], *Lauraceae*, such as the genera *Persea*, *Laurus*, for example the genera and species *Laurus nobilis* [bay], *Persea americana*, *Persea gratissima* or *Persea persea* [avocado], *Leguminosae*, such as the genus *Arachis*, for example the genus and species

Arachis hypogaea [peanut], Linaceae, such as the genera Linum, Adenolinum, for example the genera and species Linum usitatissimum, Linum humile, Linum austriacum, Linum bienne, Linum angustifolium, Linum catharticum, Linum flavum, Linum grandiflorum, Adenolinum grandiflorum, Linum lewisii, Linum narbonense, Linum perenne, Linum perenne var. lewisii, Linum pratense or Linum trigynum [linseed], Lythraeae, 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 berteriana, 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 Elaeis, 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 Chrootheca, 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 Coffea, for example the genera and species Coffea 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. glabrusculum, 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 plants are plants 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.

[0082] Preferred mosses are Physcomitrella or Ceratodon. Preferred algae are Isochrysis, Mantoniella, Ostreococcus or Cryptocodinium, and algae/diatoms such as Phaeodactylum or Thraustochytrium. More preferably, said algae or mosses are selected from the group consisting of: Shewanella, Physcomitrella, Thraustochytrium, Nannochloropsis,

Fusarium, Phytophthora, Ceratodon, Isochrysis, Aleurita, Muscarioides, Mortierella, Phaeodactylum, Cryptothecodinium, specifically from the genera and species *Thalassiosira pseudonana*, *Euglena gracilis*, *Physcomitrella patens*, *Phytophthora infestans*, *Fusarium gramineum*, *Cryptocodium cohnii*, *Ceratodon purpureus*, *Isochrysis galbana*, *Aleurita farinosa*, *Thraustochytrium* sp., *Nannochloropsis oculata*, *Muscarioides viallii*, *Mortierella alpina*, *Phaeodactylum tricornutum* or *Caenorhabditis elegans* or especially advantageously *Phytophthora infestans* and *Cryptocodium cohnii*.

[0083] Transgenic plants may be obtained by transformation techniques as elsewhere in this specification. Preferably, transgenic plants can be obtained by T-DNA-mediated transformation. Such vector systems are, as a rule, characterized in that they contain at least the *vir* genes, which are required for the *Agrobacterium*-mediated transformation, and the sequences which delimit the T-DNA (T-DNA border). Suitable vectors are described elsewhere in the specification in detail.

[0084] Also encompassed are transgenic non-human animals comprising the vector or polynucleotide of the present invention. Preferred non-human transgenic animals envisaged by the present invention are fish, such as herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna.

[0085] It will be understood that in order to produce the LCPUFA according to the invention, the C16- or C18- fatty acids must first be desaturated by the enzymatic activity of a desaturase and subsequently be elongated by at least two carbon atoms via an elongase in the non-human transgenic organism. After one elongation cycle, this enzyme activity gives C18- or C20-fatty acids and after two or three elongation cycles C22- or C24-fatty acids. The activity of the desaturases and elongases used in the process according to the invention preferably leads to C18-, C20-, C22- and/or C24-fatty acids, advantageously with at least two double bonds in the fatty acid molecule, preferably with three, four or five double bonds, especially preferably to give C20- and/or C22-fatty acids with at least two double bonds in the fatty acid molecule, preferably with three, four or five double bonds in the molecule. After a first desaturation and the elongation have taken place, further desaturation steps such as, for example, one in the delta-5 position may take place. Products of the process according to the invention which are especially preferred are DGLA, ARA, EPA DPA and/or DHA, most preferably EPA and/or DHA. Desaturases and elongases which are required for this process may not always be present naturally in the organism. Accordingly, the present invention, preferably, envisages a transgenic non-human organism which in addition to the polynucleotide of the present invention comprises polynucleotides encoding such desaturases and/or elongases as required depending on the selected organism. Preferred desaturases and/or elongases which shall be present in the organism are at least one enzyme selected from the group consisting of: Δ -4-desaturase, Δ -5-desaturase, Δ -5-elongase, Δ -6-desaturase, Δ 12-desaturase, Δ 15-desaturase, ω 3-desaturase and Δ -6-elongase. Especially preferred are the bifunctional d12d15-Desaturases d12d15Des(Ac) from *Acanthamoeba castellanii* (WO2007042510), d12d15Des(Cp) from *Claviceps purpurea* (WO2008006202) and d12d15Des(Lg)1 from *Lottia gigantea* (WO2009016202), the d12-Desaturases d12Des(Co) from *Calendula officinalis* (WO200185968), d12Des(Lb) from *Laccaria bicolor* (WO2009016202), d12Des(Mb) from *Monosiga brevicollis* (WO2009016202), d12Des(Mg) from *Mycosphaerella graminicola* (WO2009016202), d12Des(Nh) from *Nectria haematococca* (WO2009016202), d12Des(OI) from *Ostreococcus lucimarinus* (WO2008040787), d12Des(Pb) from *Phycomyces blakesleeanus* (WO2009016202), d12Des(Ps) from *Phytophthora sojae* (WO2006100241) and d12Des(Tp) from *Thalassiosira pseudonana* (WO2006069710), the d15-Desaturases d15Des(Hr) from *Helobdella robusta* (WO2009016202), d15Des(Mc) from *Microcoleus chthonoplastes* (WO2009016202), d15Des(Mf) from *Mycosphaerella fijiensis* (WO2009016202), d15Des(Mg) from *Mycosphaerella graminicola* (WO2009016202) and d15Des(Nh)2 from *Nectria haematococca* (WO2009016202), the d4-Desaturases d4Des(Eg) from *Euglena gracilis* (WO2004090123), d4Des(Tc) from *Thraustochytrium* sp. (WO2002026946) and d4Des(Tp) from *Thalassiosira pseudonana* (WO2006069710), the d5-Desaturases d5Des(OI)2 from *Ostreococcus lucimarinus* (WO2008040787), d5Des(Pp) from *Physcomitrella patens* (WO2004057001), d5Des(Pt) from *Phaeodactylum tricornutum* (WO2002057465), d5Des(Tc) from *Thraustochytrium* sp. (WO2002026946), d5Des(Tp) from *Thalassiosira pseudonana* (WO2006069710) and the d6-Desaturases d6Des(Cp) from *Ceratodon purpureus* (WO2000075341), d6Des(OI) from *Ostreococcus lucimarinus* (WO2008040787), d6Des(Ot) from *Ostreococcus tauri* (WO2006069710), d6Des(Pf) from *Primula farinosa* (WO2003072784), d6Des(Pir)_BO from *Pythium irregulare* (WO2002026946), d6Des(Pir) from *Pythium irregulare* (WO2002026946), d6Des(Plu) from *Primula luteola* (WO2003072784), d6Des(Pp) from *Physcomitrella patens* (WO200102591), d6Des(Pt) from *Phaeodactylum tricornutum* (WO2002057465), d6Des(Pv) from *Primula vialii* (WO2003072784) and d6Des(Tp) from *Thalassiosira pseudonana* (WO2006069710), the d8-Desaturases d8Des(Ac) from *Acanthamoeba castellanii* (EP1790731), d8Des(Eg) from *Euglena gracilis* (WO200034439) and d8Des(Pm) from *Perkinsus marinus* (WO2007093776), the ω 3-Desaturases ω 3Des(Pi) from *Phytophthora infestans* (WO2005083053), ω 3Des(Pir) from *Pythium irregulare* (WO2008022963), ω 3Des(Pir)2 from *Pythium irregulare* (WO2008022963) and ω 3Des(Ps) from *Phytophthora sojae* (WO2006100241), the bifunctional d5d6-elongases d5d6Elo(Om)2 from *Oncorhynchus mykiss* (WO2005012316), d5d6Elo(Ta) from *Thraustochytrium aureum* (WO2005012316) and d5d6Elo(Tc) from *Thraustochytrium* sp. (WO2005012316), the d5-elongases d5Elo(At) from *Arabidopsis thaliana* (WO2005012316), d5Elo(At)2 from *Arabidopsis thaliana* (WO2005012316), d5Elo(Ci) from *Ciona intestinalis* (WO2005012316), d5Elo(OI) from *Ostreococcus lucimarinus* (WO2008040787), d5Elo(Ot) from *Ostreococcus tauri* (WO2005012316), d5Elo(Tp) from *Thalassiosira pseudonana* (WO2005012316) and

d5Elo(XI) from *Xenopus laevis* (WO2005012316), the d6-elongases d6Elo(OI) from *Ostreococcus lucimarinus* (WO2008040787), d6Elo(Ot) from *Ostreococcus tauri* (WO2005012316), d6Elo(Pi) from *Phytophthora infestans* (WO2003064638), d6Elo(Pir) from *Pythium irregulare* (WO2009016208), d6Elo(Pp) from *Physcomitrella patens* (WO2001059128), d6Elo(Ps) from *Phytophthora sojae* (WO2006100241), d6Elo(Ps)2 from *Phytophthora sojae* (WO2006100241), d6Elo(Ps)3 from *Phytophthora sojae* (WO2006100241), d6Elo(Pt) from *Phaeodactylum tricornutum* (WO2005012316), d6Elo(Tc) from *Thraustochytrium* sp. (WO2005012316) and d6Elo(Tp) from *Thalassiosira pseudonana* (WO2005012316), the d9-elongases d9Elo(Ig) from *Isochrysis galbana* (WO2002077213), d9Elo(Pm) from *Perkinsus marinus* (WO2007093776) and d9Elo(Ro) from *Rhizopus oryzae* (WO2009016208).

[0086] Furthermore, the present invention encompasses a method for the manufacture of polyunsaturated fatty acids comprising:

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

[0087] The term "polyunsaturated fatty acids (PUFA)" as used herein refers to fatty acids comprising at least two, preferably, three, four, five or six, double bonds. Moreover, it is to be understood that such fatty acids comprise, preferably from 18 to 24 carbon atoms in the fatty acid chain. More preferably, the term polyunsaturated fatty acids relates to long chain PUFA (LCPUFA) having from 20 to 24 carbon atoms in the fatty acid chain. Preferred unsaturated fatty acids in the sense of the present invention are selected from the group consisting of arachidonic acid (ARA) 20:4 (5,8,11,14), eicosapentaenoic acid (EPA) 20:5 (5,8,11,14,17), and docosahexaenoic acid (DHA) 22:6 (4,7,10,13,16,19) and, more preferably, from EPA and DHA. Thus, it will be understood that most preferably, the methods provided by the present invention relating to the manufacture of EPA or DHA. Moreover, also encompassed are the intermediates of LCPUFA which occur during synthesis starting from oleic acid 18:1 (9), preferably, linoleic acid 18:2 (9,12), alpha-linolenic acid 18:3 (9,12,15), gamma-linolenic acid 18:3 (6,9,12), stearidonic acid 18:4 (6,9,12,15), dihomo-gamma-linoleic acid 20:3 (8,11,14), eicosadienoic acid 20:2 (11,14), eicosatrienoic acid 20:3 (11,14,17), eicosatetraenoic acid 20:4 (8,11,14,17) and docospentaenoic acid (DPA) 22:5 (4,7,10,13,16).

[0088] The term "cultivating" as used herein refers maintaining and growing the host cells under culture conditions which allow the cells to produce the said polyunsaturated fatty acid, i.e. the PUFA and/or LCPUFA referred to above, preferably, as triglyceride esters. This implies that the polynucleotide of the present invention is expressed in the host cell so that the acyltransferase activity is present. Suitable culture conditions for cultivating the host cell are described in more detail below.

[0089] The term "obtaining" as used herein encompasses the provision of the cell culture including the host cells and the culture medium as well as the provision of purified or partially purified preparations thereof comprising the polyunsaturated fatty acids, preferably, as triglyceride esters. More preferably, the PUFA and LCPUFA are to be obtained as triglyceride esters, e.g., in form of an oil. More details on purification techniques can be found elsewhere herein below.

[0090] The host cells to be used in the method of the invention are grown or cultured in the manner with which the skilled artisan is familiar, depending on the host organism. Usually, host cells are grown in a liquid medium comprising a carbon source, usually in the form of sugars, a nitrogen source, usually in the form of organic nitrogen sources such as yeast extract or salts such as ammonium sulfate, trace elements such as salts of iron, manganese and magnesium and, if appropriate, vitamins, at temperatures of between 0°C and 100°C, preferably between 10°C and 60°C under oxygen or anaerobic atmosphere dependent on the type of organism. The pH of the liquid medium can either be kept constant, that is to say regulated during the culturing period, or not. The cultures can be grown batchwise, semi-batchwise or continuously. Nutrients can be provided at the beginning of the fermentation or administered semicontinuously or continuously: The produced PUFA or LCPUFA can be isolated from the host cells as described above by processes known to the skilled artisan, e.g., by extraction, distillation, crystallization, if appropriate precipitation with salt, and/or chromatography. It might be required to disrupt the host cells prior to purification. To this end, the host cells can be disrupted beforehand. The culture medium to be used must suitably meet the requirements of the host cells in question. Descriptions of culture media for various microorganisms which can be used as host cells according to the present invention can be found in the textbook "Manual of Methods for General Bacteriology" of the American Society for Bacteriology (Washington D.C., USA, 1981). Culture media can also be obtained from various commercial suppliers. All media components are sterilized, either by heat or by filter sterilization. All media components may be present at the start of the cultivation or added continuously or batchwise, as desired. If the polynucleotide or vector of the invention which has been introduced in the host cell further comprises an expressible selection marker, such as an antibiotic resistance gene, it might be necessary to add a selection agent to the culture, such as an antibiotic in order to maintain the stability of the introduced polynucleotide. The culture is continued until formation of the desired product is at a maximum. This is normally achieved within 10 to 160 hours. The fermentation broths can be used directly or can be processed further. The biomass may, according to requirement, be removed completely or partially from the fermentation

broth by separation methods such as, for example, centrifugation, filtration, decanting or a combination of these methods or be left completely in said broth. The fatty acid preparations obtained by the method of the invention, e.g., oils, comprising the desired PUFA or LCPUFA as triglyceride esters are also suitable as starting material for the chemical synthesis of further products of interest. For example, they can be used in combination with one another or alone for the preparation of pharmaceutical or cosmetic compositions, foodstuffs, or animal feeds. Chemically pure triglycerides comprising the desired PUFA or LCPUFA can also be manufactured by the methods described above. To this end, the fatty acid preparations are further purified by extraction, distillation, crystallization, chromatography or combinations of these methods. In order to release the fatty acid moieties from the triglycerides, hydrolysis may be also required. The said chemically pure triglycerides or free fatty acids are, in particular, suitable for applications in the food industry or for cosmetic and pharmacological compositions.

[0091] Moreover, the present invention relates to a method for the manufacture of polyunsaturated fatty acids comprising:

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

[0092] Further, it follows from the above that a method for the manufacture of an oil, lipid or fatty acid composition is also envisaged by the present invention comprising the steps of any one of the aforementioned methods and the further step of formulating PUFA or LCPUFA as oil, lipid or fatty acid composition. Preferably, said oil, lipid or fatty acid composition is to be used for feed, foodstuffs, cosmetics or pharmaceuticals. Accordingly, the formulation of the PUFA or LCPUFA shall be carried out according to the GMP standards for the individual envisaged products. For example, oil may be obtained from plant seeds by an oil mill. However, for product safety reasons, sterilization may be required under the applicable GMP standard. Similar standards will apply for lipid or fatty acid compositions to be applied in cosmetic or pharmaceutical compositions. All these measures for formulating oil, lipid or fatty acid compositions as products are comprised by the aforementioned manufacture.

[0093] The term "oil" refers to a fatty acid mixture comprising unsaturated and/or saturated fatty acids which are esterified to triglycerides. Preferably, the triglycerides in the oil of the invention comprise PUFA or LCPUFA as referred to above. The amount of esterified PUFA and/or LCPUFA is, preferably, approximately 30%, a content of 50% is more preferred, a content of 60%, 70%, 80% or more is even more preferred. The oil may further comprise free fatty acids, preferably, the PUFA and LCPUFA referred to above. For the analysis, the fatty acid content can be, e.g., determined by GC analysis after converting the fatty acids into the methyl esters by transesterification. The content of the various fatty acids in the oil or fat can vary, in particular depending on the source. The oil, however, shall have a non-naturally occurring composition with respect to the PUFA and/or LCPUFA composition and content. It will be understood that such a unique oil composition and the unique esterification pattern of PUFA and LCPUFA in the triglycerides of the oil shall only be obtainable by applying the methods of the present invention specified above. Moreover, the oil of may comprise other molecular species as well. Specifically, it may comprise minor impurities of the polynucleotide or vector of the invention. Such impurities, however, can be detected only by highly sensitive techniques such as PCR.

[0094] The contents of all references cited throughout this application are herewith incorporated by reference in general and with respect to their specific disclosure content referred to above.

[0095] This invention is further illustrated by the following figures and examples which should not be construed as limiting the scope of the invention.

FIGURES

[0096]

Figure 1: Cloning strategy employed for stepwise buildup of plant expression plasmids of the invention.

Figure 2: Vector map of the *bbc* construct used for Arabidopsis transformation.

Figure 3: GC chromatogram of fatty acids methyl esters of total fatty acids of Col-0, *fae1* mutant and *fae1* transformed with *bbc*. Total fatty acids were measured as described by Wu et al., 2005. The content of the different fatty is indicated in table 5.

Figure 4: Total ion count of 26 acyl CoA ESI-MS/MS MRM pairs for Arabidopsis (A) Col-0 and (B) *fae1* harbouring EPA biosynthesis pathway. Maturing Arabidopsis seeds were harvested 18 days after flowering. Acyl-CoA was extracted according to Larson et al (2001) and LC conditions after Han et al. (2010).

Figure 5: Identification of Eicosapentaenoic and Arachidonic-CoA's in the acyl CoA pool of Arabidopsis Col-0 and EPA producing plants. MRM chromatograms of co-eluting acyl-CoA of interest in (A) wild type and (C) *fae1* harbouring EPA biosynthetic pathway with recorded reactions shown for each transition, isotopic peaks (IP) of homologous long chain acyl CoA are shown. (B) Characteristic fragmentation of the protonated acyl-CoA by neutral loss of 507 to give the protonated acyl pantetheine group.

Figure 6: LPCAT activity assay.

A yeast mutant lacking LPEAT and LPCAT activity (due to knockout of the gene YOR175c) was transformed with the empty vector pYES2.1 (lane marked "-") and with pYES2.1 harboring the cDNA of pLPAAT_c6316(No) (lane 1 and 2, SEQ-ID: 13). Microsomal isolations of these transformants and the wildtype yeast strain BY4742 (lane marked "+") containing 5µg protein where incubated with alpha-linolenic acid-CoA and [¹⁴C]-18:1-lysophosphatidylcholine (LPC). Thin layer chromatography was performed to separate lipid classes. Like for wildtype yeast (lane marked "+"), phosphatidylcholine (PC) is observed for both yeast clones shown in lane 1 and 2, indicating the gene pLPAAT_c6316(No) has LPCAT activity and complements the missing LPCAT activity of the knockout strain.

Figure 7: LPAAT activity assay.

A yeast mutant lacking LPAAT activity (due to knockout of the gene YDL052c) was transformed with the empty vector pYES2.1 (lane marked "-") and with pYES2.1 harboring the cDNA of pLPAAT_c6316(No) (lane 1 and 2, SEQ-ID: 13). Microsomal isolations of these transformants and the wildtype yeast strain BY4742 (lane marked "+") containing 5µg protein where incubated with alpha-linolenic acid-CoA and [¹⁴C]-18:1-lysophosphatidic acid (LPA). Thin layer chromatography was performed to separate lipid classes. Like for wildtype yeast (lane marked "+"), phosphatidic acid (PA) is observed for both yeast clones shown in lane 1 and 2, indicating the gene pLPAAT_c6316(No) has LPAAT activity and complements the missing LPAAT activity of the knockout strain.

Figure 8: DGAT activity assay.

A yeast mutant lacking the capability to synthesis TAG (due to knockout of the four genes YCR048W, YNR019W, YOR245C and YNR008W) was transformed with the empty vector pYES2.1 (lane marked "-") and with pYES2.1 harboring the cDNA of pDGAT2-c19425mod(Ta) (SEQ-ID 52, lane 1 and 2), pDGAT2_c4648(No) (SEQ-ID 34, lane 5 and 6), pDGAT2_c48271(No) (SEQ-ID 102, lane 7 and 8), BnDGAT1 (SEQ-ID 107, lane 9 and 10), AtDGAT1 (SEQ-ID 105, lane 11 and 12), pDGAT2_c699(No) (SEQ-ID 19, lane 13 and 14) and pDGAT2_c2959(No) (SEQ-ID 25, lane 15). Microsomal isolations of these transformants and the wildtype yeast strain G175 (lane marked "+") where incubated with ¹⁴C-labeled oleic acid and diacylglycerole (DAG). Thin layer chromatography was performed to separate lipid classes. Like for wildtype yeast (lane marked "+"), triacylglycerole (TAG) is observed in lane 1, 2, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15, indicating pDGAT2-c19425mod(Ta), pDGAT2_c4648(No), pDGAT2_c48271(No), BnDGAT1, AtDGAT1, pDGAT2_c699(No) and pDGAT2_c2959(No) encode polypeptides having DGAT activity and complement the missing TAG-synthesis capability of the knockout.

Figure 9: Substrate specificity of AtDGAT1 and BnDGAT1. The specific activity of the enzymes AtDGAT1 and BnDGAT1 using the substrates indicated at the x-axis is given as the amount (in nmol) of substrate consumed in one minute per mg total protein and was determined as described in example 10.

Figure 10: Substrate specificity of pDGAT2-c19425(Ta) compared to AtDGAT1 and BnDGAT1. The specific activity of the enzymes pDGAT2-c19425(Ta), AtDGAT1 and BnDGAT1 using the substrates indicated at the x-axis is given as the amount (in nmol) of substrate consumed in one minute per mg total protein and was determined as described in example 10.

Figure 11: Substrate specificity of pDGAT2_c699(No) and pDGAT2_c4648(No) compared to AtDGAT1 and BnDGAT1. The specific activity of the enzymes pDGAT2_c699(No) and pDGAT2_c4648(No), AtDGAT1 and BnDGAT1 using the substrates indicated at the x-axis is given as the amount (in nmol) of substrate consumed in one minute per mg total protein and was determined as described in example 10.

EXAMPLES

Example 1: General cloning methods

[0097] Cloning methods as e.g. use of restriction endonucleases to cut double stranded DNA at specific sites, agarose gel electrophoreses, purification of DNA fragments, transfer of nucleic acids onto nitrocellulose and nylon membranes, joining of DNA-fragments, transformation of E.coli cells and culture of bacteria where performed as described in Sam-

brook et al. (1989) (Cold Spring Harbor Laboratory Press: ISBN 0-87965-309-6).

Example 2: Sequence Analysis of recombinant DNA

5 **[0098]** Sequencing of recombinant DNA-molecules was performed using a laser-fluorescence DNA sequencer (Applied Biosystems Inc, USA) employing the sanger method (Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467). Expression constructs harboring fragments obtained by polymerase chain reactions were subjected to sequencing to confirm the correctness of expression cassettes consisting of promoter, nucleic acid molecule to be expressed and terminator to avoid mutations that might result from handling of the DNA during cloning, e.g. due to incorrect primers, mutations from exposure to UV-light or errors of polymerases.

Example 3: Cloning of yeast expression construct via homologous recombination

15 **[0099]** The open reading frame listed in SEQ ID NOs: 52, 1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, 55, 102, 105 and 107 encoding polypeptides with the amino acid sequence SEQ ID NOs: 53, 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 56, 103, 106 and 108 that have acyltransferase activity can be amplified using the primer listed in table 2 in a polymerase chain reaction. By doing so, the open reading frame is 5' fused to about 60 nucleotides of the 3' end of the GAL1 promoter sequence with simultaneous introduction of and Asc I and/or Nco I restriction site between the fusion site and 3' fused to about 60 nucleotides of the 5' end of the CYC1 terminator sequence with simultaneous introduction of and Pac I restriction site. To integrate these fragments into pYES2.1 TOPO downstream of the galactose inducible GAL1 Promotor via homologous recombination, the vector pYES2.1 (Invitrogen) can be digested using the restriction endonucleases Pvu II and Xba I, and Saccharomyces cerevisiae can be transformed with 5 to 20ng of linearized pYES2.1 TOPO vector and 20 to 100ng PCR product per 50 µl competent cells using the transformation method described by Schiestl et al. (Schiestl et al. (1989) Curr. Genet. 16(5-6), pp. 339-346), to obtain

25 pYES-pLPLAT_c1216(No), pYES-pLPLAT_c3052(No), pYES-pLPEAT-c7109(Ta), pYES-pLPAAT_c2283(No), pYES-pLPAAT_c6316(No), pYES-pDGAT2_irc24907(No), pYES-pDGAT2_c699(No), pYES-pDGAT2_c1910(No), pYES-pDGAT2_c2959(No), pYES-pDGAT2_c4857(No), pYES-pDGAT1_c21701(No), pYES-pDGAT2_c4648(No), pYES-pDGAT2_c1660(No), pYES-pDGAT2_c29432(No), pYES-pDGAT2_c1052(No), pYES-pDGAT2-c18182(Ta), pYES-pDGAT2-c5568(Ta), pYES-pDGAT2-c19425(Ta), pYES-pDGAT2_c48271(No), AtDGAT1, BnDGAT1 and pYES-pGPAT_c813(No) in various wildtype yeasts and yeast mutants. Positive transformants can be selected based on the complementation of the URA auxotrophy of the chosen S.cerevisia strain. To validate the correctness of the expression construct harbored by a particular yeast clone, plasmids can be isolated as described in Current Protocols in Molecular Biology (Hoffmann, Curr. Protoc. Mol. Biol. 2001 May; Chapter 13:Unit13.11), transformed into E.coli for amplification and subjected to sequencing of the expression cassette as described in example 2. For later cloning into plant expression

35 plasmids, the introduced restrictions site for Asc I and/or Nco I in combination with Pac I can be used.

Table 2: Primer sequences for cloning acyltransferase-polynucleotides of the invention for yeast expression

Gene-Name	Primer	SEQ-ID
pLPLAT_c1216(No)	Forward: ataaaagtatcaacaaaaattgtaatatac- ctctatactttaacgtcaaggagaaaaaccccg- gatcggcgcgccaccatggacaaggcactggcaccgtt	46
	Reverse: aactataaaaaataaataggaccttagacttca- ggttgctaaactcctccttttcggttagagcggatt- taattaactaaacttctcctcctcctccta	47
pLPLAT_c3052(No)	Forward: ataaaagtatcaacaaaaattgtaatatac- ctctatactttaacgtcaaggagaaaaaccccg- gatcggcgcgccaccatgaccacgactgtcatctctag	48
	Reverse: aactataaaaaataaataggaccttagacttca- ggttgctaaactcctccttttcggttagagcggatttaattaatca- aagcctcccgcaacgagc	49

EP 2 585 603 B1

(continued)

Gene-Name	Primer	SEQ-ID
pLPEAT-c7109(Ta)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctatactttaacgtcaaggagaaaaaccccg- gatcggcgcgccaccatggagggcatcgagtcgatagt	50
	Reverse: aactataaaaaataaatagggacctagacttca- ggttgtctaactcctccttttcggttagagcggatt- taattaactataaggcttctccggcgcg	51
pLPAAT_c2283(No)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctatactttaacgtcaaggagaaaaaccccg- gatcggcgcgccaccatgaagacgcccacgagcctggc	52
	Reverse: aactataaaaaataaatagggacctagacttca- ggttgtctaactcctccttttcggttagagcggatt- taattaattaagctctcgaatcgctccttct	53
pLPAAT_c6316(No)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctatactttaacgtcaaggagaaaaaccccg- gatcggcgcgccaccatggcaggaggaagatggacgt	54
	Reverse: aactataaaaaataaatagggacctagacttca- ggttgtctaactcctccttttcggttagagcggatttaattaatca- cgacgccggcgcccttgcagt	55
pDGA T2_lrc24907(N o)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctatactttaacgtcaaggagaaaaaccccg- gatcggcgcgccaccatggcaccctcccaccggcccc	56
	Reverse: aactataaaaaataaatagggacctagacttca- ggttgtctaactcctccttttcggttagagcggatttaattaatcattt- gaccactaaggtggcct	57
pDGAT2_c699(No)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctatactttaacgtcaaggagaaaaaccccg- gatcggcgcgccaccatgggtctatttggcagcgggat	58
	Reverse: aactataaaaaataaatagggacctagacttca- ggttgtctaactcctccttttcggttagagcggatt- taattaactaaaagaattcaacgtccgat	59

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EP 2 585 603 B1

(continued)

Gene-Name	Primer	SEQ-ID
pDGAT2_c1910(No)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctatactttaacgtcaaggagaaaaaaccccg- gatcggcgcgccaccatggtgagatccccgagtcgtc	60
	Reverse: aactataaaaaataaataggacctagacttca- ggttgctaaactcctccttttcggttagagcggatt- taattaactaaaagaaatccagctccctgt	61
pDGAT2_c2959(No)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctatactttaacgtcaaggagaaaaaaccccg- gatcggcgcgccaccatgacgccgaagccgatcac	62
	Reverse: aactataaaaaataaataggacctagacttca- ggttgctaaactcctccttttcggttagagcggatttaattaact- caatggacaacgggcgcg	63
pDGAT2_c4857(No)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctatactttaacgtcaaggagaaaaaaccccg- gatcggcgcgccaccatggcttacctctccgctcgtcg	64
	Reverse: aactataaaaaataaataggacctagacttca- ggttgctaaactcctccttttcggttagagcggatttaattaat- taggcgatcgcaatgaactcct	65
pDGAT1_c21701(No)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctatactttaacgtcaaggagaaaaaaccccg- gatcggcgcgccaccatgccttttgacgggctgcatc	66
	Reverse: aactataaaaaataaataggacctagacttca- ggttgctaaactcctccttttcggttagagcggatttaattaatcac- ccgaaaatacctcctct	67
pDGAT2_c4648(No)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctatactttaacgtcaaggagaaaaaaccccg- gatcggcgcgccaccatggccaaggctaacttcccgcc	68
	Reverse: aactataaaaaataaataggacctagacttca- ggttgctaaactcctccttttcggttagagcggatttaattaact- tataagcagcttctgt	69

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EP 2 585 603 B1

(continued)

Gene-Name	Primer	SEQ-ID
pDGA T2_c1660(No)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctataactttaacgtcaaggagaaaaaaccccg- gatcggcgcgccaccatggtggtgcagggattaagctg	70
	Reverse: aactataaaaaataaataggacctagacttca- ggttgctaaactcctccttttcggttagagcggatttaattaatca- caacaggaccaatttatgat	71
pDGAT2_c29432(No)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctataactttaacgtcaaggagaaaaaaccccg- gatcggcgcgccaccatggtgatggcgccgctcgcgggcg	72
	Reverse: aactataaaaaataaataggacctagacttca- ggttgctaaactcctccttttcggttagagcggatttaattaatca- gacgatgcaagcgtctgt	73
pDGAT2_c1052(No)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctataactttaacgtcaaggagaaaaaaccccg- gatcggcgcgccaccatgggcgctaccactgcgacca	74
	Reverse: aactataaaaaataaataggacctagacttca- ggttgctaaactcctccttttcggttagagcggatttaattaatca- cgactcggacagtccaaaa	75
pDGA T2-c18182(Ta)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctataactttaacgtcaaggagaaaaaaccccg- gatcggcgcgccaccatgctggtcgttgagcacagcgc	76
	Reverse: aactataaaaaataaataggacctagacttca- ggttgctaaactcctccttttcggttagagcggatt- taattaactacacaaatcgcacgtctgt	77
pDGAT2-c5568(Ta)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctataactttaacgtcaaggagaaaaaaccccg- gatcggcgcgccaccatggtctcctctgccttccta	78
	Reverse: aactataaaaaataaataggacctagacttca- ggttgctaaactcctccttttcggttagagcggatt- taattaactacgagtcagccacttgatgc	79

EP 2 585 603 B1

(continued)

Gene-Name	Primer	SEQ-ID
pDGA T2-c19425(Ta)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctatactttaacgtcaaggagaaaaaaccg- gatcggcgcgccaccatgttcttcgcatcgaacggga	80
	Reverse: aactataaaaaataaataggacctagacttca- ggttgctaaactcctccttttcggttagagcggatttaattaactaac- cctcgggttacagcgccg	81
pGPAT_c813(No)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctatactttaacgtcaaggagaaaaaaccg- gatcggcgcgccaccatgccatcccgcagcaccattga	82
	Reverse: aactataaaaaataaataggacctagacttca- ggttgctaaactcctccttttcggttagagcggatttaattaatca- gacaagctccttccccct	83
pDGAT2_c48271(No)	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctatactttaacgtcaaggagaaaaaaccg- gatcggcgcgccaccatggccgcatctcaccgcgcaa	109
	Reverse: aactataaaaaataaataggacctagacttca- ggttgctaaactcctccttttcggttagagcggatttaattaactac- cacacctcaacttcgccc	110
AtDGAT1	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctatactttaacgtcaaggagaaaaaaccg- gatcggcgcgccaccatggcgatttggattctgctgg	111
	Reverse: aactataaaaaataaataggacctagacttca- ggttgctaaactcctccttttcggttagagcggatttaattaatcat- gacatcgatccttttcggt	112
BnDGAT1	Forward: ataaaagtatcaacaaaaaattgtaatatac- ctctatactttaacgtcaaggagaaaaaaccg- gatcggcgcgccaccatggagatttggattctggagg	113
	Reverse: aactataaaaaataaataggacctagacttca- ggttgctaaactcctccttttcggttagagcggatttaattaactat- gacatccttcttgcggt	114

EP 2 585 603 B1

Table 3: Coding polynucleotide sequences, amino acid sequences encoded thereby and expressed sequences (mRNA) of the acyltransferases of the invention

Gene name	Organism	ORF in bp	SEQ-ID No.	Amino acids	SEQ-ID No.	mRNA in bp	SEQ-ID No.
pLPLAT_c1216 (No)	Nannochloropsis oculata	1485	1	494	2	1908	3
pLPLAT_c3052 (No)	Nannochloropsis oculata	1776	4	591	5	2247	6
pLPEAT-c7109(Ta)	Thraustochytrium aureum	1134	7	377	8	1288	9
pLPAAT_c2283 (No)	Nannochloropsis oculata	1284	10	427	11	1826	12
pLPAAT_c6316 (No)	Nannochloropsis oculata	1395	13	464	14	1771	15
pDGAT2_lrc24907 (No)	Nannochloropsis oculata	1026	16	341	17	1100	18
pDGAT2_c699(No)	Nannochloropsis oculata	1206	19	401	20	1772	21
pDGAT2_c1910(N o)	Nannochloropsis oculata	1173	22	390	23	1239	24
pDGAT2_c2959(N o)	Nannochloropsis oculata	1089	25	362	26	1609	27
pDGAT2_c4857(N o)	Nannochloropsis oculata	1464	28	487	29	1682	30
pDGAT1_c21701 (No)	Nannochloropsis oculata	1539	31	512	32	1904	33
pDGAT2_c4648(N o)	Nannochloropsis oculata	1083	34	360	35	1362	36
pDGAT2_c1660(N o)	Nannochloropsis oculata	1695	37	564	38	2074	39
pDGAT2_c29432 (No)	Nannochloropsis oculata	1029	40	342	41	1585	42
pDGAT2_c1052(N o)	Nannochloropsis oculata	1251	43	416	44	1923	45
pDGAT2-c18182 (Ta)	Thraustochytrium aureum	930	46	309	47	1134	48
pDGAT2-c5568 (Ta)	Thraustochytrium aureum	1179	49	392	50	1303	51
pDGAT2-c19425 (Ta)	Thraustochytrium aureum	1389	52	462	53	1547	54
pGPAT_c813(No)	Nannochloropsis oculata	1977	55	658	56	2460	57
pDGAT2_c48271 (No)	Nannochloropsis oculata	960	102	319	103	1265	104

Example 4: Assembly of genes required for PUFA synthesis within a T-plasmid

[0100] For synthesis of EPA in Arabidopsis seeds, the set of genes encoding the proteins of the metabolic EPA pathway

EP 2 585 603 B1

(table 4) was combined with expression elements (promoter, terminators) and transferred into binary t-plasmids that were used for agrobacteria mediated transformation of plants as described in example 5. To this end, the general cloning strategy depicted in figure 1 was employed: Genes listed in table 4 were PCR-amplified using Phusion™ High-Fidelity DNA Polymerase (NEB, Frankfurt, Germany) according to the manufactures instructions from cDNA using primer introducing a *Nco* I and/or *Asc* I restriction site at the 5' terminus, and a *Pac* I restriction site at the 3' terminus (figure 1B). To obtain the final expression modules, PCR-amplified genes were cloned between promoter and terminator via *Nco* I and/or *Pac* I restriction sites (figure 1C). Up to three of those expression modules were combined as desired to expression cassettes harbored by either one of pENTR/A, pENTR/B or pENTR/C (figure 1D).. Finally, the Multisite Gateway™ System (Invitrogen) was used to combine three expression cassette harbored by pENTR/A, pENTR/B and pENTR/C (figure 1E) to obtain the final binary T-plasmids bbc (SEQ-ID 101, figure 2).

Table 4: Genes of the bbc construct for synthesis of EPA (20:5n-3) in Arabidopsis seeds. The elements controlling the expression of the respective genes are as well indicated.

Name	Source Organism	Activity	SEQ-ID	Promoter	Terminator
d12Des(Ps)	<i>Phytophthora sojae</i>	d-12 Desaturase	96	p-BnNapin	t-E9
d6Des(Ot)	<i>Ostreococcus tauri</i>	d-6 Desaturase	97	p-SBP	t-CatpA
d5Des(Tc)	<i>Traustochytrium ssp.</i>	d-5 Desaturase	98	p-LuCnl	t-AgroOCS
d6Elo(Pp)	<i>Phycomitrella patens</i>	d-6 Elongase	99	p-VfUSP	t-CaMV35S
o-3Des(Pi)	<i>Phytophthora infestans</i>	o-3 Desaturase	100	p-Napin	t-E9

Example 5: Plant Transformation

[0101] The resulting binary vector bbc harboring the genes reconstituting EPA biosynthesis pathway were transformed into *Agrobacterium tumefaciens* (Hofgen and Willmitzer (1988) Nucl. Acids Res. 16: 9877). The transformation of *A. thaliana* was accomplished by means of the floral-dip method (Clough and Bent (1998) Plant Journal 16: 735-743), this method is known to the skilled person. Wild-type *Arabidopsis* seeds contain considerable amounts of eicosenoic acid (20:1) (Table 5). Biosynthesis of 20:1 competes for the substrates of the PUFA biosynthesis pathway. This competition was circumvented by transforming bbc into the *Arabidopsis fae1* mutant (James et al., (1995) The Plant Cell 7:309-319).

Example 6: Quantification of metabolic fatty Acyl-CoAs in wild-type and EPA producing *Arabidopsis* seeds

[0102] The selected transgenic *Arabidopsis* plants from example 3 were analyzed in respect to PUFA content in seeds. Seeds from wild-type, *fae1* mutant and transgenics harboring the bbc construct were harvested 18 days after flowering. Total fatty acid, representing the fatty acids esterified to CoA, on lipids and as triacyl-glycerides were extracted and analyzed by gas chromatography as described in Wu et al., (2005) Nature Biotechnology 23(8): 1013-1017.

[0103] In seeds of *fae1* transformed with bbc the EPA accumulation was 12.2 %, the seeds contained small amounts of intermediate or side products: ARA (3.2 %), SDA (0.8 %), GLA (2.6 %) which were not present in wild-type or *fae1* (Fig 3, Table 5).

Table 5: Content of fatty acids in seeds of wild-type (Col-0), *fae1* mutant and *fae1* transformed with bbc construct

Fatty acid	Common name of FA	Col-0	<i>fae1</i>	<i>bbc fae1</i>
16:0	Palmitic acid	6,2	8,8	6,8
18:0	Stearic acid	3,1	4,1	5,3
18:1	Oleic acid	16,3	27,5	18,9
18:2	Linoleic acid	28,2	39,0	30,8
18:3n6	Gamma-Linolenic acid	0,0	0,0	2,6
18:3n3	Alpha-Linoleic acid	15,6	18,4	11,9
18:4n3	Stearidonic acid	0,0	0,0	0,8
20:1	Eicosenoic acid	22,8	0,4	0,3

EP 2 585 603 B1

(continued)

Fatty acid	Common name of FA	Col-0	<i>fae1</i>	<i>bbc fae1</i>
20:4n6	Arachidonic acid	0,0	0,0	3,2
20:5n3	Eicosapentaenoic acid	0,0	0,0	12,2
Others		7,8	1,8	7,2

[0104] For PUFA biosynthesis the acyl-moiety has to be shuffled between different metabolic pools. For example, the elongation of the acyl chain by two carbon atoms occurs specifically on acyl-CoA (Zank et al., (2002) The Plant Journal 318(3):255-268. The efficiency of the transfer of the acyl-residue between the metabolic pools may represent a bottleneck for PUFA production in plants. Therefore the accumulation of EPA or intermediates of EPA biosynthesis as CoA species was analyzed by LC/MS². As a control CoA pool of wild-type seeds were as well analyzed. The Acyl-CoA metabolites were extracted from the seed tissue according to Larson and Graham, 2001. LC/MS² was applied as described by Magnes et al., 2005. Briefly, CoA were separated with high resolution by reversed-phase high performance liquid chromatography (HPLC) with a ammonium hydroxide and acetonitrile gradient. The acyl-CoA species were identified and quantified by multireaction monitoring using triple quadrupole mass spectrometry. Only a few methods using mass spectrometry for characterization of long chain acyl-CoA have been published, the majority of which employ negative ionisation mode showing abundant ions. In contrast, positive ionisation has only one abundant ion [M - H]⁺, furthermore the predominant ion in MS² spectra is the fatty acyl-pantetheine fragment (m/z 507 - Fig 5 B), characteristic of CoA-activated substances. In choosing the acyl-pantetheine of interest in multireaction monitoring mode (MRM) a very sensitive, selective and reproducible method was established. CoA-activated substances can be monitored by scanning for the neutral loss of phosphoadenosine diphosphate. Generally for reliable analysis, all interfering peaks must be chromatographically separated; in the case of EPA and ARA this is not possible (Fig 4 B). However through the use of MRM, incorporating very short dwell times (15 ms), it is possible to follow the individual chromatograms of acyl-CoA of interest and demonstrate the presence of EPA and ARA in the acyl CoA pool (Fig 5 C). According to internal standards the eicosapentaenoyl-CoA was in the range of ...% of the total Co-A pool.

[0105] In conclusion these results show that PUFA accumulate in the metabolic CoA pool and are not transferred to DAG to be released as TAG into the seed oil. Such a bottleneck may be overcome by the co-expression of an acyltransferase from table 3, having the appropriate substrate specificity. The application of suitable acyltransferase may increase the flux of fatty acid between the metabolic pools and increase the PUFA biosynthesis rate.

Example 7: Activity assays using yeast extracts

[0106] To characterize the functions of the acyltransferase polypeptides of the invention, yeast mutants can be employed that are defective in certain acyltransferase activities. For example, the yeast mutant Y13749 (Genotype: BY4742; Mat alpha; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; YDL052c::kanMX4) lacking LPAAT activity can be transformed with expression constructs harboring candidate polypeptides to check for restoration (complementation) of LPAAT activity, the yeast mutant Y12431 (genotype BY4742; Mat alpha; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; YOR175c::kanMX4) lacking LPLAT activity can be transformed with expression constructs harboring candidate polypeptides to check for restoration (complementation) of LPLAT activity, the yeast mutant H1246 (genotype MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 YOR245::KanMX4 YNR008W::TRP1 YCR048W::HIS3 YNR019W::LEU2) lacking the ability to synthesize triacylglycerole can be transformed with expression constructs harboring candidate polypeptides to check for restoration (complementation) of the ability to synthesis triacylglycerole. The yeast mutants can for example harbor the expression constructs listed in example 3 employing the transformation method described in example 3.

[0107] For LPAAT activity assay, clones of the yeast mutant Y13749 harboring pYES-pLPAAT_c6316(No) can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The next day, expression of the acyltransferase polypeptide can be induced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of OD₆₀₀=0.1. Cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspended in 1 ml resuspension buffer (25 mM Tris/HCL pH 7.6) and disrupted using acid washed zirconium bead (200µm average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant is transferred to a fresh tube and centrifuged at 3000 x g for 5 min. The obtained supernatant is the crude extract. Protein content is measured according to Bradford (Bradford, M.M. (1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. Assay mixtures contain 1 to 50 µg of protein, 10 µl of 100 nM [¹⁴C]-18:1-LPA (giving about 2000 dpm/nmol), 10 µl of 50 nM 18:1-CoA or 50nM 18:3n-3-CoA in assay buffer (25mM Tris/HCL pH 7.6, 0.5 mg/ml BSA) to give a total volume of 100 µl. Samples are incubated for 10 min at 30°C. The assays are terminated by extraction of the lipids into chloroform according to Blight

and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911-917). Lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using chloroform/methanol/acetic acid/water (90:15:10:3), and autoradiographic pictures are taken using an instant imager (Packard). It can be seen by the formation of phosphatidic acid (PA) in figure 7, that pLPAAT_c6316(No) (SEQ-ID 13, lane 1 and 2) encodes a polypeptide having LPAAT activity.

[0108] For LPCAT and LPEAT activity assay, clones of the yeast mutant Y12431 harboring pYES-pLPAAT_c6316(No) can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The next day, expression of the acyltransferase polypeptide can be induced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of OD₆₀₀=0.1. Cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspended in 1 ml resuspension buffer (25 mM Tris/HCL pH 7.6) and disrupted using acid washed zirconium bead (200µm average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant is transferred to a fresh tube and centrifuged at 3000 x g for 5 min. The obtained supernatant is the crude extract. Protein content is measured according to Bradford (Bradford, M.M. (1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. Assay mixtures contain either 10µl 100nM [¹⁴C]-LPC (LPCAT activity assay) or 10µl 100nM [¹⁴C]-LPE (LPEAT activity assay), 1 to 50 µg of protein, 10 µl of 50nM 18:1-CoA or 50nM 18:3n-3-CoA in assay buffer (25mM Tris/HCL pH 7.6, 0.5 mg/ml BSA) to give a total volume of 100 µl. Samples are incubated for 10 min at 30°C. The assays are terminated by extraction of the lipids into chloroform according to Bligh and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911-917). Lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using chloroform/methanol/acetic acid/water (90:15:10:3), and autoradiographic pictures are taken using an instant imager (Packard). It can be seen by the formation of phosphatidylethanolamine (PE) in figure 6, that pLPAAT_c6316(No) (SEQ-ID 13, lane 1 and 2) encodes a polypeptide having LPCAT activity.

[0109] For DGAT activity assay, clones of the yeast mutant H1246 harboring either one of pYES-pDGAT2_c699(No), pYES-pDGAT2_c2959(No), pYES-pDGAT2_c4648(No), pYES-pDGAT2_c48271(No), pYES-pDGAT2-c19425(Ta), pYES-AtDGAT1, or pYES-BnDGAT1 can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The next day, expression of the acyltransferase polypeptides can be induced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of OD₆₀₀=0.1. Activity as indicated by the formation of TAG (as indicated, the mutant H1246 is unable to synthesize TAG) can be measured either by relying on yeast-endogenous substrate-DAG, or by providing substrate in an *in vitro* assay.

For the former type of assay, cells are harvested after reaching stationary phase during incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspended in 2 ml resuspension buffer (phosphate buffered saline (PBS) pH 7.4, see Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989). The equivalent of 200 mg cell pellet is taken, the volume adjusted to 210µl using PBS and 790 µl of methanol:chloroform (2:1) are added. Cells are disrupted using acid washed zirconium bead (200µm average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm and lipids are extracted according to Bligh and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911-917).

The *in vitro* assay is the preferred way of testing for DGAT activity, when activity is known or expected to be weak when relying on endogenous substrate. Instead, both the type and concentration of the DAG acceptor molecule, as well as the type and concentration of the fatty acid-CoA can be controlled. To do so, cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspended in 1 ml resuspension buffer (25 mM Tris/HCL pH 7.6) and disrupted using acid washed zirconium bead (200µm average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant is transferred to a fresh tube and centrifuged at 3000 x g for 5 min. The obtained supernatant is the crude extract. Protein content is measured according to Bradford (Bradford, M.M.(1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. Assay mixtures contain 10 µl 50 nM [¹⁴C]-6:0-DAG (giving about 3000 dpm/nmol), 50 µg of microsomal protein (the amount can be adjusted to stay within linear conditions without substrate limitation), 10 µl of 50 nM 18:3n-3-CoA or 50 nM 22:6n-3-CoA in assay buffer (50 mM Hepes buffer pH 7.2, 1 mg/ml BSA) to give a total volume of 100 µl. Samples are incubated for 10 min at 30°C.

In either case - *in vivo* or *in vitro* assay - lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using hexane:diethylether:acetic acid (70:30:1), and stained in iodine vapor. It can be seen by the formation of triacylglycerole (TAG) using the *in vitro* assay-conditions in figure 8, that pDGAT2-c19425mod(Ta) (SEQ-ID 52, lane 1 and 2), pDGAT2_c4648(No) (SEQ-ID 34, lane 5 and 6), pDGAT2_c48271(No) (SEQ-ID 102, lane 7 and 8), BnDGAT1 (SEQ-ID 107, lane 9 and 10), AtDGAT1 (SEQ-ID 105, lane 11 and 12), pDGAT2_c699(No) (SEQ-ID 19, lane 13 and 14) and pDGAT2_c2959(No) (SEQ-ID 25, lane 15) encode polypeptides having DGAT activity.

[0110] Table 6 summarizes the results of the LPCAT, LPAAT and DGAT activity tests.

EP 2 585 603 B1

Table 6: Measured with microsomal protein and [14C]-18:1-LPA, [14C]-18:1-LPC or [14C]-6:0-1,2-DAG. Of the in vitro DGAT assay, 1 mg/ml of BSA was added to reduce activity for staying in the linear range.

Enzyme Class	Candidate	SEQ-IDs (ORF / protein / mRNA)	Activity in vitro using 18:3-CoA nmol/(mg*min)	Activity in vitro using 22:6-CoA nmol/(mg*min)	Activity in vivo
LPAAT	pLPAAT_c6316 (No)	13/14/15	81	64	
LPCAT	pLPAAT_c6316 (No)	13/14/15	38	9	
DGAT	pDGAT2_c699 (No)	19/20/21	0,22	0,17	Yes
DGAT	pDGAT2_c2959 (No)	25/26/27	0,95	0,67	Yes
DGAT	pDGAT2_c4648 (No)	34/35/36	1,4	0,17	Yes
DGAT	pDGAT2_c48271 (No)	102/103/104	1,6	0	Yes
DGAT	pDGAT2-c19425 (Ta)	52/53/54	4,0	5,6	Yes
DGAT	AtDGAT1	105/106/--	1,6	1,2	Yes
DGAT	BnDGAT1	107/108/--	2,4	1,5	Yes

Example 8: Determination of substrate specificity for LPAAT

[0111] For determination of substrate specificities of the LPAAT enzymes, clones of the yeast mutant Y13749 (described in example 7) harboring LPAAT genes in the pYES plasmid can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The next day, expression of the acyltransferase polypeptides can be induced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of OD₆₀₀=0.1. Cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspended in 1 ml disruption buffer (20 mM Tris/HCL pH 7.6, 10 mM MgCl₂, 1 mM EDTA, 5% glycerol, 0.3 M (NH₄)₂SO₄) and disrupted using acid washed zirconium beads (200µm average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant and the beads are transferred to a fresh tube. Disruption buffer is added up to 20 ml and the tube is centrifuged at 8000 x g for 5 min. The obtained supernatant is centrifuged for 2 hrs at 42000 rpm at 4°C. The pellet (microsomal fraction) is resuspended in a small volume of 0.1 M phosphate buffer pH 7.2. Protein content in the microsomal fraction is measured according to Bradford (Bradford, M.M. (1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. Assay mixtures contain 1-5 µg of microsomal protein (the amount is adjusted to achieve linear conditions without substrate limitation), 10 µl of 1 mM [¹⁴C]-18:1-LPA (5000 dpm/nmol), 10 µl of 1 mM acyl-CoA in assay buffer (0.1 M phosphate buffer pH 7.2., 10 mg/ml Bovine Serum Albumine (BSA)) to give a total volume of 100 µl. Like to amount of microsomal protein added to the assay, also the amount of BSA has influence on observed enzyme activities, where higher amounts of BSA result on lower activities and lower amounts of BSA result in higher activities. The enzyme specificity can be tested for different acyl-CoA:s, e.g. 14:0-CoA, 16:0-CoA, 18:1-CoA, 18:2-CoA, 18:3-CoA, γ18:3-CoA, 18:4-CoA, 20:3-CoA, 20:4-CoA, 20:4(n-3)-CoA, 20:5-CoA, 22:5-CoA, 22:6-CoA. Samples are incubated for 4 min at 30°C. The assays are terminated by extraction of the lipids into chloroform according to Bligh and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911-917). Lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using chloroform/methanol/acetic acid/water (90:15:10:3), and autoradiographic pictures are taken using an instant imager (Packard). The amount of phosphatidic acid (PA) produced in the reaction (and hence the enzyme activity) can be determined from the picture.

Example 9: Determination of substrate specificity for LPLAT

[0112] For LPCAT and LPEAT activity assay, clones of the yeast mutant Y12431 harboring LPLAT genes in the pYES plasmid can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The

next day, expression of the acyltransferase polypeptides can be induced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of $OD_{600}=0.1$. Cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspended in 1 ml disruption buffer (20 mM Tris/HCL pH 7.6, 10 mM $MgCl_2$, 1 mM EDTA, 5% glycerol, 0.3 M $(NH_4)_2SO_4$) and disrupted using acid washed zirconium beads (200 μ m average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant and the beads are transferred to a fresh tube. Disruption buffer is added up to 20 ml and the tube is centrifuged at 8000 x g for 5 min. The obtained supernatant is centrifuged for 2 hrs at 42000 rpm at 4°C. The pellet (microsomal fraction) is resuspended in a small volume of 0.1 M phosphate buffer pH 7.2. Protein content in the microsomal fraction is measured according to Bradford (Bradford, M.M. (1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. Assay mixtures contain either 10 μ l 1 mM [^{14}C]-18:1-Lysophosphatidylcholine (-LPC), 5000 dpm/nmol (LPCAT assay) or 10 μ l 1 mM [^{14}C]-18:1-Lysophosphatidylethanolamine (-LPE), 5000 dpm/nmol (LPEAT assay), 1-10 μ g of microsomal protein (the amount is adjusted to achieve linear conditions without substrate limitation), 10 μ l of 1 mM acyl-CoA in assay buffer (0.1 M phosphate buffer pH 7.2., 10 mg/ml BSA) to give a total volume of 100 μ l. Like to amount of microsomal protein added to the assay, also the amount of BSA has influence on observed anzyme activities, where higher amounts of BSA result on lower activities and lower amounts of BSA result in higher activities. The enzyme specificity can be tested for different acyl-CoA:s, e.g. 14:0-CoA, 16:0-CoA, 18:1-CoA, 18:2-CoA, 18:3-CoA, γ 18:3-CoA, 18:4-CoA, 20:3-CoA, 20:4-CoA, 20:4(n-3)-CoA, 20:5-CoA, 22:5-CoA, 22:6-CoA.. Samples are incubated for 4 min at 30°C. The assays are terminated by extraction of the lipids into chloroform according to Bligh and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911-917). Lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using chloroform/methanol/acetic acid/water (90:15:10:3), and autoradiographic pictures are taken using an instant imager (Packard). The amount of phosphatidyl choline (PC) or phosphatidyl ethanol amine (PE) produced in the reaction (and hence the enzyme activity) can be determined from the picture.

Example 10: Determination of substrate specificity for DGAT

[0113] For DGAT activity assay, clones of the yeast mutant H1246 harboring either one of pYES-pDGAT2_c699(No), pYES-pDGAT2_c2959(No), pYES-pDGAT2_c4648(No), pYES-pDGAT2_c48271(No), pYES-pDGAT2-c19425(Ta), pYES-AtDGAT1, or pYES-BnDGAT1 can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The next day, expression of the acyltransferase polypeptides can be induced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of $OD_{600}=0.1$. Cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspended in 1 ml disruption buffer (20 mM Tris/HCL pH 7.6, 10 mM $MgCl_2$, 1 mM EDTA, 5% glycerol, 0.3 M $(NH_4)_2SO_4$) and disrupted using acid washed zirconium beads (200 μ m average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant and the beads are transferred to a fresh tube. Disruption buffer is added up to 20 ml and the tube is centrifuged at 8000 x g for 5 min. The obtained supernatant is centrifuged for 2 hrs at 42000 rpm at 4°C. The pellet (microsomal fraction) is resuspended in a small volume of 0.1 M phosphate buffer pH 7.2. Protein content in the microsomal fraction is measured according to Bradford (Bradford, M.M. (1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. Assay mixtures contain 5 μ l 1 mM [^{14}C]-6:0-DAG, 3000 dpm/nmol, 1-100 μ g of microsomal protein (the amount is adjusted to achieve linear conditions without substrate limitation), 5 μ l of 1 mM acyl-CoA in assay buffer (50 mM Hepes buffer pH 7.2, 1 mg/ml BSA) to give a total volume of 100 μ l. The enzyme specificity can be tested for different acyl-CoA:s, e.g. 14:0-CoA, 16:0-CoA, 18:1-CoA, 18:2-CoA, 18:3-CoA, γ 18:3-CoA, 18:4-CoA, 20:3-CoA, 20:4-CoA, 20:4(n-3)-CoA, 20:5-CoA, 22:5-CoA, 22:6-CoA.. Samples are incubated for 4 min at 30°C. The assays are terminated by extraction of the lipids into chloroform according to Bligh and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911-917). Lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using hexane:diethylether:acetic acid (70:30:1), and autoradiographic pictures are taken using an instant imager (Packard). The amount of triacylglycerol (TAG) produced in the reaction (and hence the enzyme activity) can be determined from the picture. In Brassica napus and Arabidopsis, the DGAT involved in TAG-formation in seeds are of the DGAT1 type. The enzyme activity AtDGAT1 and BnDGAT1 for the different substrates can be seen in figure 9. The enzyme activity of pDGAT2-c19425(Ta) for the different substrates, compared to AtDGAT1 and BnDGAT1 is shown in figure 10. The enzyme activity of pDGAT2_c699(No) and pDGAT2_c4648(No) for the different substrates, compared to AtDGAT1 and BnDGAT1 is shown in figure 11. The data in figure 10 and 11 show clearly, that all DGAT2 enzymes shown in these figures vary strongly towards their activities for the various substrates, whereas the DGAT1 involved in TAG formation in Arabidopsis and Brassica napus exhibit less variability towards these different substrates.

Example 11: Determination of substrate selectivity for LPAAT

[0114] For determination of substrate selectivities of the LPAAT enzymes, clones of the yeast mutant Y13749 (de-

EP 2 585 603 B1

scribed in example 7) harboring LPAAT genes can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The next day, expression of the acyltransferase polypeptides can be induced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of $OD_{600}=0.1$. Cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspended in 1 ml disruption buffer (20 mM Tris/HCL pH 7.6, 10 mM $MgCl_2$, 1 mM EDTA, 5% glycerol, 0.3 M $(NH_4)_2SO_4$) and disrupted using acid washed zirconium beads (200 μ m average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant and the beads are transferred to a fresh tube. Disruption buffer is added up to 20 ml and the tube is centrifuged at 8000 x g for 5 min. The obtained supernatant is centrifuged for 2 hrs at 42000 rpm at 4°C. The pellet (microsomal fraction) is resuspended in a small volume of 0.1 M phosphate buffer pH 7.2. Protein content in the microsomal fraction is measured according to Bradford (Bradford, M.M. (1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. The substrate selectivity can be determined by mixing equimolar amounts of different acyl-CoA:s in the same reaction and measure the preference for using the different acyl groups as substrates. The assay is run as in the specificity studies (Example 5) but scaled up 18 times to get sufficient amount of PA for detection. Up to 4 different acyl-CoA:s can be used in the assay in equimolar amount instead of one single acyl-CoA. Samples are incubated for 4 min at 30°C. The assays are terminated by extraction of the lipids into chloroform according to Bligh and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911-917). Lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using chloroform/methanol/acetic acid/water (90:15:10:3), and autoradiographic pictures are taken using an instant imager (Packard). The phosphatidic acid (PA) is recovered from the plate and the fatty acids methylated in situ on the gel with sulphuric acid (2%) in methanol. Fatty acid methyl esters are extracted with hexane and separated by gas-liquid chromatography (GLC) using a WCOT fused silica 50 m \times 0.32 mm ID capillary column coated with CP-Wax 58-CB DF = 0.3 (Chrompack inc., The Netherlands) and quantified relative to methyl-heptadecanoate added as an internal standard. The selectivity can be determined by calculating the amount of each acyl group that has been acylated to LPA.

Example 12: Determination of substrate selectivity for LPLAT

[0115] For LPCAT and LPEAT activity assay, clones of the yeast mutant Y12431 harboring LPLAT genes can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The next day, expression of the acyltransferase polypeptides can be induced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of $OD_{600}=0.1$. Cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspended in 1 ml disruption buffer (20 mM Tris/HCL pH 7.6, 10 mM $MgCl_2$, 1 mM EDTA, 5% glycerol, 0.3 M $(NH_4)_2SO_4$) and disrupted using acid washed zirconium beads (200 μ m average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant and the beads are transferred to a fresh tube. Disruption buffer is added up to 20 ml and the tube is centrifuged at 8000 x g for 5 min. The obtained supernatant is centrifuged for 2 hrs at 42000 rpm at 4°C. The pellet (microsomal fraction) is resuspended in a small volume of 0.1 M phosphate buffer pH 7.2. Protein content in the microsomal fraction is measured according to Bradford (Bradford, M.M. (1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. The substrate selectivity can be determined by mixing equimolar amounts of different acyl-CoA:s in the same reaction and measure the preference for using the different acyl groups as substrates. The assay is run as in the specificity studies (Example 6) but scaled up 18 times to get sufficient amount of PC or PE for detection. Up to 4 different acyl-CoA:s can be used in the assay in equimolar amount instead of one single acyl-CoA. Samples are incubated for 4 min at 30°C. The assays are terminated by extraction of the lipids into chloroform according to Bligh and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911-917). Lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using chloroform/methanol/acetic acid/water (90:15:10:3), and autoradiographic pictures are taken using an instant imager (Packard). The PC or PE is recovered from the plate and the fatty acids methylated in situ on the gel with sulphuric acid (2%) in methanol. Fatty acid methyl esters are extracted with hexane and separated by gas-liquid chromatography (GLC) using a WCOT fused silica 50 m \times 0.32 mm ID capillary column coated with CP-Wax 58-CB DF = 0.3 (Chrompack inc., The Netherlands) and quantified relative to methyl-heptadecanoate added as an internal standard. The selectivity can be determined by calculating the amount of each acyl group that has been acylated to LPC or LPE.

Example 13: Determination of substrate selectivity for DGAT

[0116] For DGAT activity assay, clones of the yeast mutant H1246 harboring DGAT genes can be grown at 28°C in 10ml selective media (SC-URA) with 2% raffinose as carbon source over night. The next day, expression of the acyltransferase polypeptides can be induced by transferring the cells to fresh media containing 2% galactose, for example by inoculating 100 ml of fresh culture to an optical density (measure at 600nm) of $OD_{600}=0.1$. Cells are harvested after 24h incubation at 28°C by centrifugation at 3000 x g for 5 min and resuspended in 1 ml disruption buffer (20 mM Tris/HCL

EP 2 585 603 B1

pH 7.6, 10 mM MgCl₂, 1 mM EDTA, 5% glycerol, 0.3 M (NH₄)₂SO₄) and disrupted using acid washed zirconium beads (200µm average diameter) in a mill (Retsch, Germany) by three minutes agitation at 300rpm. The supernatant and the beads are transferred to a fresh tube. Disruption buffer is added up to 20 ml and the tube is centrifuged at 8000 x g for 5 min. The obtained supernatant is centrifuged for 2 hrs at 42000 rpm at 4°C. The pellet (microsomal fraction) is resuspended in a small volume of 0.1 M phosphate buffer pH 7.2. Protein content in the microsomal fraction is measured according to Bradford (Bradford, M.M. (1976), Anal. Biochem. Bd. 72, pp. 248-254) with bovine serum albumin as standard. The substrate selectivity can be determined by mixing equimolar amounts of different acyl-CoA:s in the same reaction and measure the preference for using the different acyl groups as substrates. The assay is run as in the specificity studies (Example 7) but scaled up 18 times to get sufficient amount of TAG for detection. Up to 4 different acyl-CoA:s can be used in the assay in equimolar amount instead of one single acyl-CoA. Samples are incubated for 4 min at 30°C. The assays are terminated by extraction of the lipids into chloroform according to Bligh and Dyer (Bligh, E.G. and Dyer, W.J. (1959), Can. J. Biochem. Physiol. 37, pp. 911-917). Lipids are separated on thin layer chromatography (TLC) silica 60 plates (Merck) using chloroform/methanol/acetic acid/water (90:15:10:3), and autoradiographic pictures are taken using an instant imager (Packard). The TAG is recovered from the plate and the fatty acids methylated in situ on the gel with sulphuric acid (2%) in methanol. Fatty acid methyl esters are extracted with hexane and separated by gas-liquid chromatography (GLC) using a WCOT fused silica 50 m×0.32 mm ID capillary column coated with CP-Wax 58-CB DF = 0.3 (Chrompack inc., The Netherlands) and quantified relative to methyl-heptadecanoate added as an internal standard. The selectivity can be determined by calculating the amount of each acyl group that has been acylated to TAG.

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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55 <213> Thraustochytrium aureum

<400> 7

EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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5 Glu Cys Gln Pro Val Tyr Val Ala Asn His Thr Ser Met Ile Asp Val
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10 Ile Ile Leu Gln Gln Met Arg Cys Phe Ser Leu Val Gly Gln Arg His
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Lys Gly Ile Val Arg Phe Leu Gln Glu Val Val Leu Gly Cys Leu Gln
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15 Cys Val Trp Phe Asp Arg Gly Glu Ile Lys Asp Arg Ala Ala Val Ala
 165 170 175

20 Arg Lys Leu Asn Glu His Ala Asn Asp Pro Thr Arg Asn Pro Leu Leu
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Val Phe Pro Glu Gly Thr Cys Val Asn Asn Glu Tyr Val Ile Gln Phe
 195 200 205

25 Lys Lys Gly Ile Phe Glu Ile Gly Ala Pro Val Val Pro Val Ala Ile
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30 Lys Tyr Asn Lys Met Phe Val Asp Pro Phe Trp Asn Ser Arg Ala Gln
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35 Ser Phe Pro Met His Leu Val Glu Leu Met Thr Ser Trp Cys Leu Ile
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Cys Glu Val Trp Tyr Leu Lys Pro Leu Glu Arg Met Glu Arg Glu Ser
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40 Ser Thr Asp Phe Ala Ala Arg Val Lys Lys Ala Ile Ala Asp Gln Ala
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45 Gly Leu Lys Asn Val Asn Trp Asp Gly Tyr Met Lys Tyr Trp Lys Pro
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Ser Glu Arg Tyr Leu Arg Ala Arg Gln Ala Ile Phe Ala Lys Thr Leu
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50 Arg Lys Ile His Ser Arg Ser Leu Glu Gln Asp Lys Ala Asp Arg Gln
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55 Ala Ile Leu His Asp Leu Asp Gly Ala Phe Pro Asp Ser Gly Thr His
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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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<212> DNA

<213> Nannochloropsis oculata

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EP 2 585 603 B1

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EP 2 585 603 B1

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<400> 17

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EP 2 585 603 B1

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 70 Pro Val Tyr Ala Phe Gly Glu Asn Asp Leu Tyr Arg Thr Phe Asn His
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EP 2 585 603 B1

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<212> DNA

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<400> 18

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EP 2 585 603 B1

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EP 2 585 603 B1

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10 Ser Asn Leu Tyr His Thr Ile Thr Trp Gly Arg Lys Thr Arg Leu Ala
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<400> 21

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

<212> DNA

<213> Nannochloropsis oculata

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<213> Nannochloropsis oculata

EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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<213> Nannochloropsis oculata

<400> 30

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EP 2 585 603 B1

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EP 2 585 603 B1

<213> Nannochloropsis oculata

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 <213> Nannochloropsis oculata

EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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5 Thr Arg Ala Leu Met Pro Val Ala Leu Pro Arg Glu Glu Gly Asp Pro
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15 Val Gly Val Lys Gly Ser Leu Glu Arg Leu His Ala Ala Asn Ala Thr
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25 Ala Phe Asp Ser Phe Val Arg His Thr Phe Val Phe Ser Asn Val Pro
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30 Tyr Met Ala Phe Ala Asn Leu Ile Pro Gln Val Gly Ala Leu Ser Leu
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35 Asn Gly Lys Ile Phe Thr Cys Leu Val Leu Asp Asp Glu Val Thr Pro
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 <212> DNA
 <213> Nannochloropsis oculata

50 <400> 33

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EP 2 585 603 B1

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EP 2 585 603 B1

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<212> DNA

55 <213> Nannochloropsis oculata

<400> 34

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 <213> Nannochloropsis oculata

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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<212> DNA
<213> Nannochloropsis oculata

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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Val Leu Met Ile Val Ser Leu His Phe Tyr Met Pro Thr Thr Thr Thr
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Val Glu Glu Met Met Val Gly Lys Glu Gly Val Gly Glu Glu Asp Glu
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Glu Met Val Glu Glu Lys Val Asp Val Thr Thr Ala Ala Thr Thr Asn
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25 Ala Leu Leu Arg Thr Glu Lys Gln Arg Leu Leu Leu Ala Lys Glu Ser
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Ala Thr Thr Thr Thr Thr Thr Ala Thr Val Thr Thr Gly Gln Thr Ser
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Lys Thr Ser Thr Ser Phe Met Pro Val Arg Val Asp Glu Ala Ser Leu
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35 Glu Gln Phe Arg Arg Leu Thr Val Ile Thr Val Leu Ser Asn Met Gln
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Tyr Leu Pro Phe Leu Leu Pro Ile Leu Pro Phe Val Leu Ser Gly Leu
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Pro Leu Pro Val Ala Ser Phe His Trp Phe Gly Ala Phe Cys Cys Leu
195 200 205

45 Thr Ser Ala Val Val Leu Asn Ala Tyr Val Lys Thr Thr Leu Ala Lys
210 215 220

Ala Gly Asn Arg Ile Ser Ser Phe Gln Arg Ser Leu Leu Asn Val Leu
225 230 235 240
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Pro Thr Leu Ile Tyr Ala Ala Pro Gly Leu Ile Cys Phe Phe Ala Trp
245 250 255
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Ser Gln His Gln Gly Gly Arg Glu Asp Gly Lys Glu Arg Ala Val Thr

EP 2 585 603 B1

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EP 2 585 603 B1

Glu Glu Val Arg Lys Leu Ser Lys Lys Tyr Phe Glu Ser Ile Gln Glu
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<210> 39

<211> 2074

<212> DNA

<213> Nannochloropsis oculata

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<400> 39

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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<212> PRT

40 <213> Nannochloropsis oculata

<400> 41

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EP 2 585 603 B1

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 Ser Arg Pro Ala Ser Trp Arg Thr Thr Pro Met Val Val Gly Gly Ser
 35 40 45
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 Leu Leu Val Val Gly Ser Phe Val Trp Val Pro Leu Val Ile Trp Leu
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 Gly Trp Lys Lys Cys Arg Thr Arg Asn Arg Arg Ile Val Tyr Val Leu
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 Val Leu Cys Val Ile Leu Thr Leu Pro Thr Arg Arg Trp Asp Ala Val
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 Val Leu Asn Gly Leu Trp Ser Arg Phe Val Glu Tyr Phe Ser Val Gln
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 Val Val Gly Asp Asp Pro Leu Pro Lys Asp Arg Ser Ala Val Tyr Ala
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 Val Ile Pro His Gly Thr Phe Pro Phe Gly Leu Gly Val Val Ser Leu
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 Gly Pro Leu Asn Lys Ile Phe Asn Lys Val Arg Pro Val Val Ala Ser
 145 150 155 160
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 Ala Val Leu Arg Phe Pro Gly Phe Gly Gln Leu Ile Gly Phe Ala Gly
 165 170 175
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 Gly Val Asp Ala Gly Pro Lys Glu Val Ser Lys Ala Ile Lys Lys Gly
 180 185 190
 Cys Ser Val Ser Ile Cys Pro Gly Gly Ile Ala Glu Met Phe Trp Gly
 195 200 205
 Phe Pro Lys Glu Gly Cys Leu Pro Arg Glu Glu Tyr Ala Phe Leu Gln
 210 215 220

EP 2 585 603 B1

Ser Arg Lys Gly Phe Ile Arg Met Ala Met Lys His Asn Val Pro Val
 225 230 235 240
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 Val Pro Val Tyr Cys Phe Gly Asn Thr His Ala Met His Lys Ala Lys
 245 250 255
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 Thr Pro Trp Val Leu Glu Ala Leu Ser Arg Leu Leu Lys Thr Ser Leu
 260 265 270
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 Ile Leu Thr Trp Gly Arg Trp Gly Leu Pro Ile Pro Tyr Arg Val Pro
 275 280 285
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 Leu Leu Tyr Ala Val Gly Lys Pro Leu Arg Leu Leu His Ala Glu Asn
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 Pro Thr Pro Ala Gln Ile Glu Ala Ala His Ala Glu Phe Cys Arg Ala
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EP 2 585 603 B1

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 55 <212> DNA
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 <400> 43

EP 2 585 603 B1

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<212> PRT

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EP 2 585 603 B1

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10 Asp Gly Ala Ala Gly Asp Ala Thr Ala Gly Gly Leu Ser Arg Ser Thr
 35 40 45

15 Pro Thr Ala Ala Pro Glu Ala Ser Thr Ser Leu Ser Ser Arg Leu Val
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20 Pro Ser Pro Ala Gln Val Ser Ser Met Pro Pro Ala Gln Ala Ser Ala
 65 70 75 80

25 Thr Pro Ile Val Val Arg Pro Glu Ala Arg Pro Ala Gly Pro Gln Gly
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30 Arg Leu Gln Ala Leu Gly Ala Val Leu Phe Leu Gly Leu Met Gly Ser
 100 105 110

35 Ser Leu Tyr Leu Val Ile Ala Ser Ala Leu Tyr Ile Val Ile Gly Phe
 115 120 125

40 Gly Val Leu Gly His Arg Ile Cys Pro Ser Ile Leu Leu Gly Val Trp
 130 135 140

45 Val Gly Gln Ala Leu Ile Ser Val Lys Val Leu His Gln Asp Pro Glu
 145 150 155 160

50 Gly Ile Lys Arg Ser Trp Leu Phe Arg Glu Met Val Asn Phe Phe Asp
 165 170 175

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EP 2 585 603 B1

Val Thr Leu Val Met Glu Gln Lys Leu Asp Thr Ser Lys Lys Tyr Leu
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195 200 205

10 Ala Tyr Phe Val Ser Asp Val Val Pro Gly Gly Gly Lys Ile Phe Cys
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Leu Ile His Ser Gly Ile Phe His Leu Pro Ile Val Arg Phe Phe Met
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15 Gly Glu Trp Gly Ala Leu Ser Ala Asn Lys Glu Ser Val Ala Glu Ala
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20 Lys Gln Gln Gly Gln His Cys Ser Ile Val Val Gly Gly Val Ala Glu
260 265 270

25 Ile Phe Leu Gln Asn Gly Glu Thr Glu Gln Leu Gln Leu Arg Lys Gly
275 280 285

30 Phe Ile Arg Glu Ala Leu Arg Asn Gly Tyr Asp Leu Val Pro Met Phe
290 295 300

35 His Phe Gly Ala Thr Arg Met Tyr His Phe Val Gly Pro Val Ser Phe
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Trp Arg Ser Leu Ser Asn Tyr Leu Pro Phe Pro Phe Phe Leu Ile Gly
325 330 335

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45 Ile Ala Val Gly Ser Pro Ile Gly Leu Ala Ala Leu Tyr Gly Val Pro
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Glu Gly Gln Ser Val Pro Asp Pro Asp Leu Ala Lys Val Asp Leu Ile
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EP 2 585 603 B1

<212> DNA

<213> *Nannochloropsis oculata*

<400> 45

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EP 2 585 603 B1

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EP 2 585 603 B1

ccc

1923

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 <213> Thraustochytrium aureum

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EP 2 585 603 B1

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15 Leu Ala Leu His Pro Ile Pro Asp Ile Ser Asp Ala Val Tyr Ser Ser
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EP 2 585 603 B1

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Leu Glu Leu Gly Asn Thr Val Gly Leu Val Gly Asp Gly Ile Ala Gly
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Ile Phe Gln Cys Asp His Asn Asp Glu Val Val Ala Leu Arg Thr Arg
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30 Lys Gly Leu Ala Lys Leu Ala Leu Arg Thr Gly Arg Pro Val Leu Pro
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Cys Tyr Ser Leu Gly Asn Thr Glu Ala Phe Ser Val Trp Phe Asp Arg
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Trp Gly Val Met Glu Arg Leu Ser Arg Lys Leu Gln Ala Ser Val Phe
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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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15 Ser Leu Trp Arg Ser His Phe Pro His Leu Ser Val Asn Pro Leu Thr
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Ala Ser Ile Ile His Phe Val Pro Val Met Arg Asp Val Leu Gln Trp
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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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EP 2 585 603 B1

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5 Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp Met Val Arg
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15 Ile Ile Leu Ala Phe Leu Val Ser Ala Val Phe His Glu Leu Cys Ile
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EP 2 585 603 B1

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Claims

1. A polynucleotide comprising a nucleic acid sequence selected from the group consisting of:
- a) a nucleic acid sequence consisting of the nucleotide sequence as shown in any one of SEQ ID NOs: 52, 7, 46 and 49;
 - b) a nucleic acid sequence encoding a polypeptide having an amino acid sequence as shown in any one of SEQ ID NOs: 53, 8, 47 and 50;
 - c) a nucleic acid sequence being at least 60% identical to the nucleic acid sequence as shown in any one of SEQ ID NOs: 52, 7, 46 and 49 or encoding a polypeptide having an amino acid sequence at least 60% identical to in any one of SEQ ID NOs: 53, 8, 47 and 50,
- and wherein said nucleic acid sequence of (a) to (c) encodes a polypeptide having acyltransferase activity.
2. The polynucleotide of claim 1, wherein said polynucleotide further comprises an expression control sequence operatively linked to the said nucleic acid sequence.
3. The polynucleotide of claim 1 or 2, wherein said polynucleotide further comprises a terminator sequence operatively linked to the nucleic acid sequence.
4. A vector comprising the polynucleotide of any one of claims 1 to 3.
5. A host cell comprising the vector of claim 4, or a plant cell, an insect cell, bacterium or algae cell comprising the polynucleotide of any one of claims 1 to 3 or the vector of claim 4.
6. A method for the manufacture of a polypeptide consisting of an amino acid sequence as shown in any one of SEQ ID NOs: 53, 8, 47 and 50 or consisting of an amino acid sequence at least 60% identical to any one of SEQ ID NOs: 53, 8, 47 and 50; and wherein said polypeptide has acyltransferase activity comprising
- a) cultivating the cell of claim 5 under conditions which allow for the production of the said polypeptide; and
 - b) obtaining the polypeptide from the cell of step a).
7. A polypeptide
- (a) encoded by nucleic acid sequence as shown in any one of SEQ ID NOs: 52, 7, 46 and 49 or being at least 60% identical to the nucleic acid sequence as shown in any one of SEQ ID NOs: 52, 7, 46 and 49, or
 - (b) consisting of an amino acid sequence as shown in any one of SEQ ID NOs: 53, 8, 47 and 50 or consisting of an amino acid sequence at least 60% identical to any one of SEQ ID NOs: 53, 8, 47 and 50,
- and wherein said polypeptide has acyltransferase activity
8. A plant, bacterium or algae comprising the polynucleotide of any one of claims 1 to 3 or the vector of claim 4.
9. The plant of claim 8, which is a plant part, or plant seed.
10. A method for the manufacture of polyunsaturated fatty acids comprising:
- a) cultivating the cell of claim 5 under conditions which allow for the production of polyunsaturated fatty acids in said host cell; and
 - b) obtaining said polyunsaturated fatty acids from the said cell.

11. A method for the manufacture of polyunsaturated fatty acids comprising:

- 5 a) cultivating the plant, bacterium or algae of claim 8 or 9 under conditions which allow for the production of polyunsaturated fatty acids in said organism; and
b) obtaining said polyunsaturated fatty acids from the said plant, bacterium or algae.

12. A method for the manufacture of an oil, lipid or fatty acid composition comprising the steps of the method of claim 10 or 11 and the further step of formulating the polyunsaturated fatty acid as oil, lipid or fatty acid composition.

10 13. The method of claim 12, wherein said oil, lipid or fatty acid composition is to be used for feed, foodstuffs, cosmetics or pharmaceuticals.

14. The method of any one of claims 10 to 13, wherein the organism is a plant or algae.

15 15. An antibody which specifically binds to a polypeptide consisting of an amino acid sequence as shown in any one of SEQ ID NOs: 53, 8, 47 and 50 or to a polypeptide consisting of an amino acid sequence at least 60% identical to any one of SEQ ID NOs: 53, 8, 47 and 50; and wherein said polypeptide has acyltransferase activity.

20 **Patentansprüche**

1. Polynukleotid umfassend eine Nukleinsäuresequenz, die aus der Gruppe bestehend aus den Folgenden ausgewählt ist:

- 25 a) einer Nukleinsäuresequenz, die aus der in einer der SEQ ID NO: 52, 7, 46 und 49 gezeigten Nukleotidsequenz besteht;
b) einer Nukleinsäuresequenz, die für ein Polypeptid mit einer Aminosäuresequenz gemäß einer der SEQ ID NO: 53, 8, 47 und 50 codiert;
30 c) einer Nukleinsäuresequenz, die zumindest zu 60% mit der Nukleinsäuresequenz gemäß einer der SEQ ID NO: 52, 7, 46 und 49 identisch ist oder die für ein Polypeptid mit einer Aminosäuresequenz, die zumindest zu 60% zu einer der SEQ ID NO: 53, 8, 47 und 50 identisch ist, codiert,

und wobei die Nukleinsäuresequenz von (a) bis (c) für ein Polypeptid mit Acyltransferaseaktivität codiert.

35 2. Polynukleotid nach Anspruch 1, wobei das Polynukleotid weiterhin eine Expressionskontrollsequenz in operativer Verknüpfung mit der Nukleinsäuresequenz umfasst.

3. Polynukleotid nach Anspruch 1 oder 2, wobei das Polynukleotid weiterhin eine Terminatorsequenz in operativer Verknüpfung mit der Nukleinsäuresequenz umfasst.

40 4. Vektor, der das Polynukleotid nach einem der Ansprüche 1 bis 3 umfasst.

5. Wirtszelle, die den Vektor nach Anspruch 4 umfasst, oder Pflanzenzelle, Insektenzelle, Bakterium oder Algenzelle, die das Polynukleotid nach einem der Ansprüche 1 bis 3 oder den Vektor nach Anspruch 4 umfasst.

45 6. Verfahren zur Herstellung eines Polypeptids, das aus einer Aminosäuresequenz nach einer der SEQ ID NO: 53, 8, 47 und 50 besteht oder aus einer Aminosäuresequenz, die zumindest zu 60% zu einer der SEQ ID NO: 53, 8, 47 und 50 identisch ist, besteht; und wobei das Polypeptid Acyltransferaseaktivität aufweist, wobei das Verfahren Folgendes umfasst:

- 50 a) Kultivieren der Zelle nach Anspruch 5 unter Bedingungen, die die Produktion des Polypeptids gestatten; und
b) Gewinnen des Polypeptids aus der Zelle gemäß Schritt a).

7. Polypeptid,

- 55 (a) das von einer Nukleinsäuresequenz gemäß einer der SEQ ID NO: 52, 7, 46 und 49 codiert wird oder das zumindest zu 60% zu der Nukleinsäuresequenz gemäß einer der SEQ ID NO: 52, 7, 46 und 49 identisch ist, oder
(b) das aus einer Aminosäuresequenz gemäß einer der SEQ ID NO: 53, 8, 47 und 50 besteht oder das aus

EP 2 585 603 B1

einer Aminosäuresequenz, die zumindest zu 60% zu einer der SEQ ID NO: 53, 8, 47 und 50 identisch ist, besteht, und wobei das Polypeptid Acyltransferaseaktivität aufweist.

- 5 8. Pflanze, Bakterium oder Alge, die/das das Polynukleotid nach einem der Ansprüche 1 bis 3 oder den Vektor nach Anspruch 4 umfasst.
9. Pflanze nach Anspruch 8, wobei es sich um einen Pflanzenteil oder einen Pflanzensamen handelt.
- 10 10. Verfahren zur Herstellung von mehrfach ungesättigten Fettsäuren, das Folgendes umfasst:
- a) Kultivieren der Zelle nach Anspruch 5 unter Bedingungen, die die Produktion von mehrfach ungesättigten Fettsäuren gestatten; und
 - b) Gewinnen der mehrfach ungesättigten Fettsäuren aus der Zelle.
- 15 11. Verfahren zur Herstellung von mehrfach ungesättigten Fettsäuren, das Folgendes umfasst:
- a) Kultivieren der Pflanze, des Bakteriums oder der Alge nach Anspruch 8 oder 9 unter Bedingungen, die die Produktion von mehrfach ungesättigten Fettsäuren in dem Organismus gestatten; und
 - b) Gewinnen der mehrfach ungesättigten Fettsäuren aus der Pflanze, dem Bakterium oder der Alge.
- 20 12. Verfahren zur Herstellung einer Öl-, Lipid- oder Fettsäurezusammensetzung, umfassend die Schritte des Verfahrens nach Anspruch 10 oder 11 und den weiteren Schritt des Formulierens der mehrfach ungesättigten Fettsäure als Öl-, Lipid- oder Fettsäurezusammensetzung.
- 25 13. Verfahren nach Anspruch 12, wobei die Öl-, Lipid- oder Fettsäurezusammensetzung für Futtermittel, Nahrungsmittel, Kosmetika oder Pharmazeutika bestimmt ist.
- 30 14. Verfahren nach einem der Ansprüche 10 bis 13, wobei es sich bei dem Organismus um eine Pflanze oder Alge handelt.
- 35 15. Antikörper, der spezifisch an ein Polypeptid, das aus einer Aminosäuresequenz gemäß einer der SEQ ID NO: 53, 8, 47 und 50 besteht, bindet oder der an ein Polypeptid, das aus einer Aminosäuresequenz, die zumindest zu 60% zu einer der SEQ ID NO: 53, 8, 47 und 50 identisch ist, besteht, bindet; und wobei das Polypeptid Acyltransferaseaktivität aufweist.

Revendications

- 40 1. Polynucléotide comprenant une séquence d'acide nucléique choisie dans le groupe constitué par :
- a) une séquence d'acide nucléique constituée par la séquence nucléotidique telle qu'illustrée selon l'une quelconque parmi SEQ ID n° 52, 7, 46 et 49 ;
 - b) une séquence d'acide nucléique codant pour un polypeptide ayant une séquence d'acides aminés telle qu'illustrée selon l'une quelconque parmi SEQ ID n° 53, 8, 47 et 50 ;
 - c) une séquence d'acide nucléique étant identique à au moins 60% par rapport à la séquence d'acide nucléique telle qu'illustrée selon l'une quelconque parmi SEQ ID n° 52, 7, 46 et 49, ou codant pour un polypeptide ayant une séquence d'acides aminés étant identique à au moins 60% par rapport à l'une quelconque parmi SEQ ID n° 53, 8, 47 et 50 ;
- 50 et où ladite séquence d'acide nucléique de (a) à (c) code pour un polypeptide ayant une activité d'acyltransférase.
- 55 2. Polynucléotide selon la revendication 1, où ledit polynucléotide comprend en outre une séquence de contrôle d'expression liée de manière opérante à ladite séquence d'acide nucléique.
3. Polynucléotide selon la revendication 1 ou 2, où ledit polynucléotide comprend en outre une séquence de terminaison liée de manière opérante à la séquence d'acide nucléique.

EP 2 585 603 B1

4. Vecteur comprenant le polynucléotide selon l'une quelconque des revendications 1 à 3.
5. Cellule hôte comprenant le vecteur selon la revendication 4, ou une cellule végétale, une cellule d'insecte, une bactérie ou une cellule algale, comprenant le polynucléotide selon l'une quelconque des revendications 1 à 3, ou le vecteur selon la revendication 4.
6. Méthode de préparation d'un polypeptide constitué de la séquence d'acides aminés telle qu'illustrée selon l'une quelconque parmi SEQ ID n° 53, 8, 47 et 50, ou constitué d'une séquence d'acides aminés étant identique à au moins 60% par rapport à l'une quelconque parmi SEQ ID n° 53, 8, 47 et 50, et où ledit polypeptide possède une activité d'acyltransférase, comprenant
- a) la culture de la cellule selon la revendication 5 dans des conditions permettant la production dudit polypeptide ;
et
b) l'obtention du polypeptide à partir de la cellule de l'étape a).
7. Polypeptide
- (a) codé par une séquence d'acide nucléique telle qu'illustrée selon l'une quelconque parmi SEQ ID n° 52, 7, 46 et 49, ou étant identique à au moins 60% par rapport à la séquence d'acide nucléique telle qu'illustrée selon l'une quelconque parmi SEQ ID n° 52, 7, 46 et 49, ou
- (b) étant constitué d'une séquence d'acides aminés telle qu'illustrée selon l'une quelconque parmi SEQ ID n° 53, 8, 47 et 50 ou constitué d'une séquence d'acides aminés identique à au moins 60% par rapport à l'une quelconque parmi SEQ ID n° 53, 8, 47 et 50 ;
- et où ledit polypeptide possède une activité d'acyltransférase.
8. Plante, bactérie ou algue, comprenant le polynucléotide selon l'une quelconque des revendications 1 à 3, ou le vecteur selon la revendication 4.
9. Plante selon la revendication 8, qui est une partie de plante, ou une semence issue d'une plante.
10. Méthode de préparation d'acides gras polyinsaturés, comprenant :
- a) la culture de la cellule selon la revendication 5 dans des conditions qui permettent la production d'acides gras polyinsaturés dans ladite cellule hôte ; et
b) l'obtention desdits acides gras polyinsaturés à partir de ladite cellule.
11. Méthode de préparation d'acides gras polyinsaturés, comprenant :
- a) la culture de la plante, bactérie ou algue selon la revendication 8 ou 9 dans des conditions qui permettent la production d'acides gras polyinsaturés dans ledit organisme ; et
b) l'obtention desdits acides gras polyinsaturés à partir de ladite plante, bactérie ou algue.
12. Méthode de préparation d'une composition d'huile, de lipides ou d'acides gras, comprenant les étapes de la méthode selon la revendication 10 ou 11, et l'étape supplémentaire consistant à formuler l'acide gras polyinsaturé sous forme de composition d'huile, de lipides ou d'acides gras.
13. Méthode selon la revendication 12, dans laquelle ladite composition d'huile, de lipides ou d'acides gras est prévue pour être utilisée dans les aliments pour animaux, les produits alimentaires, les produits cosmétiques ou les produits pharmaceutiques.
14. Méthode selon l'une quelconque des revendications 10 à 13, dans laquelle l'organisme est une plante ou une algue.
15. Anticorps se fixant spécifiquement à un polypeptide constitué d'une séquence d'acides aminés telle qu'illustrée selon l'une quelconque parmi SEQ ID n° 53, 8, 47 et 50 ou à un polypeptide constitué d'une séquence d'acides aminés identique à au moins 60% par rapport à l'une quelconque parmi SEQ ID n° 53, 8, 47 et 50 ;
et où ledit polypeptide possède une activité d'acyltransférase.

Fig 1:

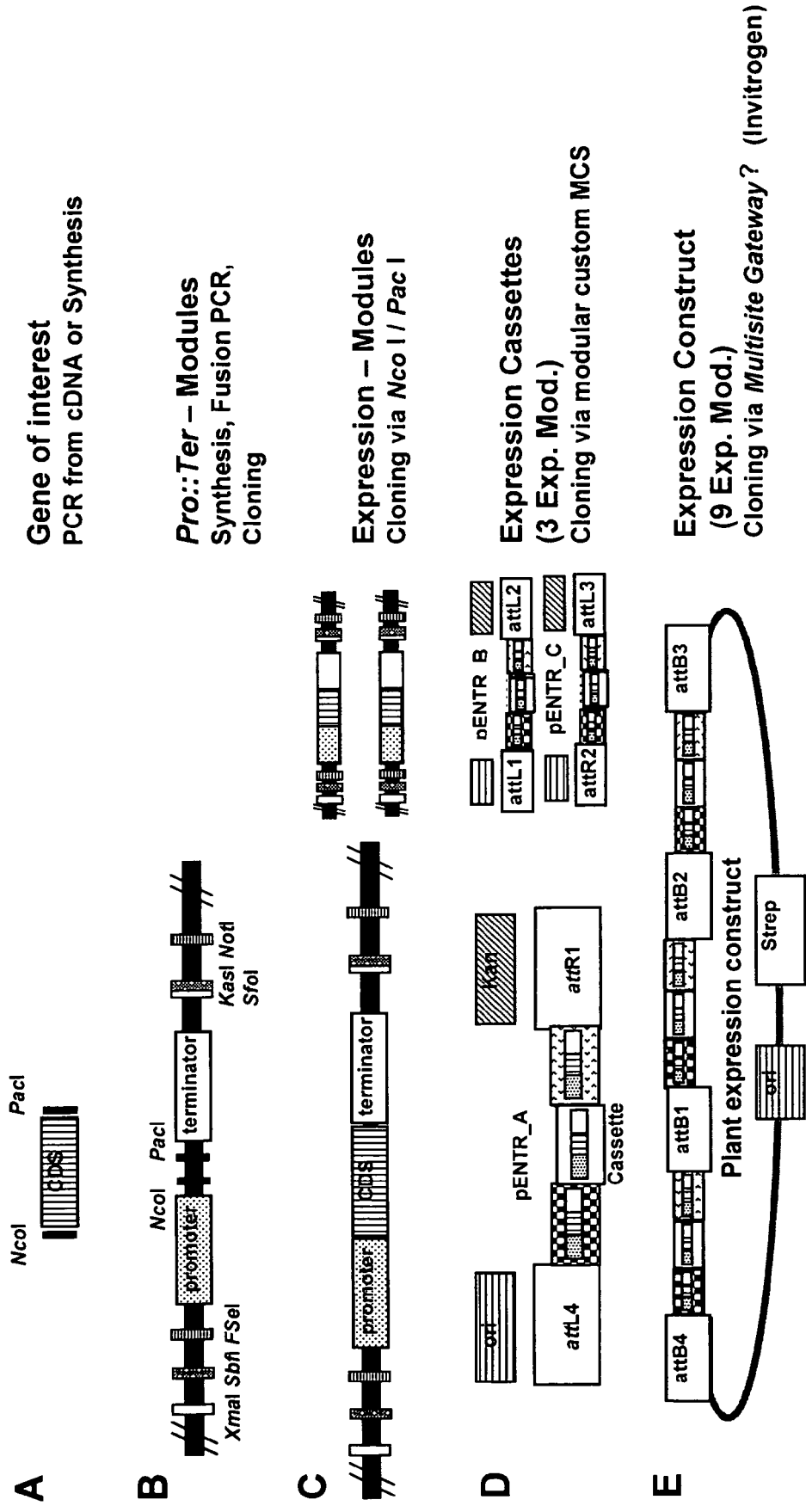


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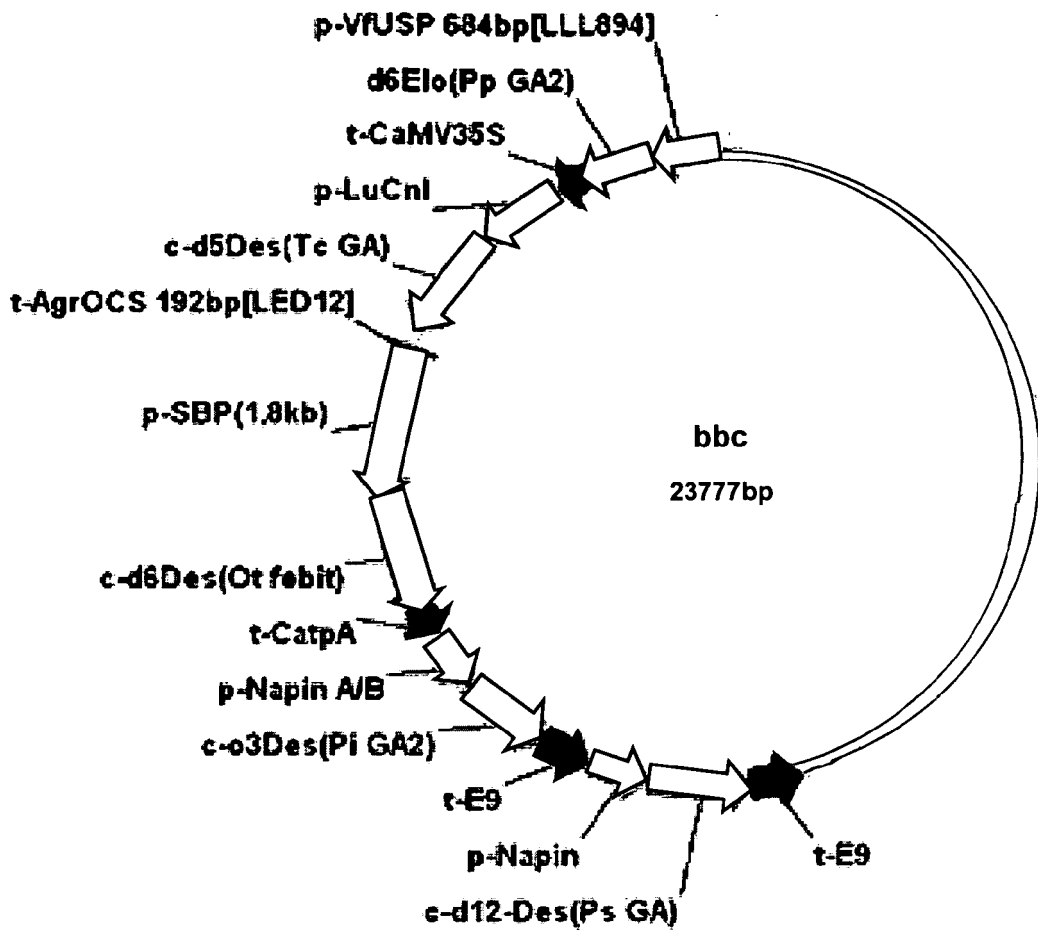
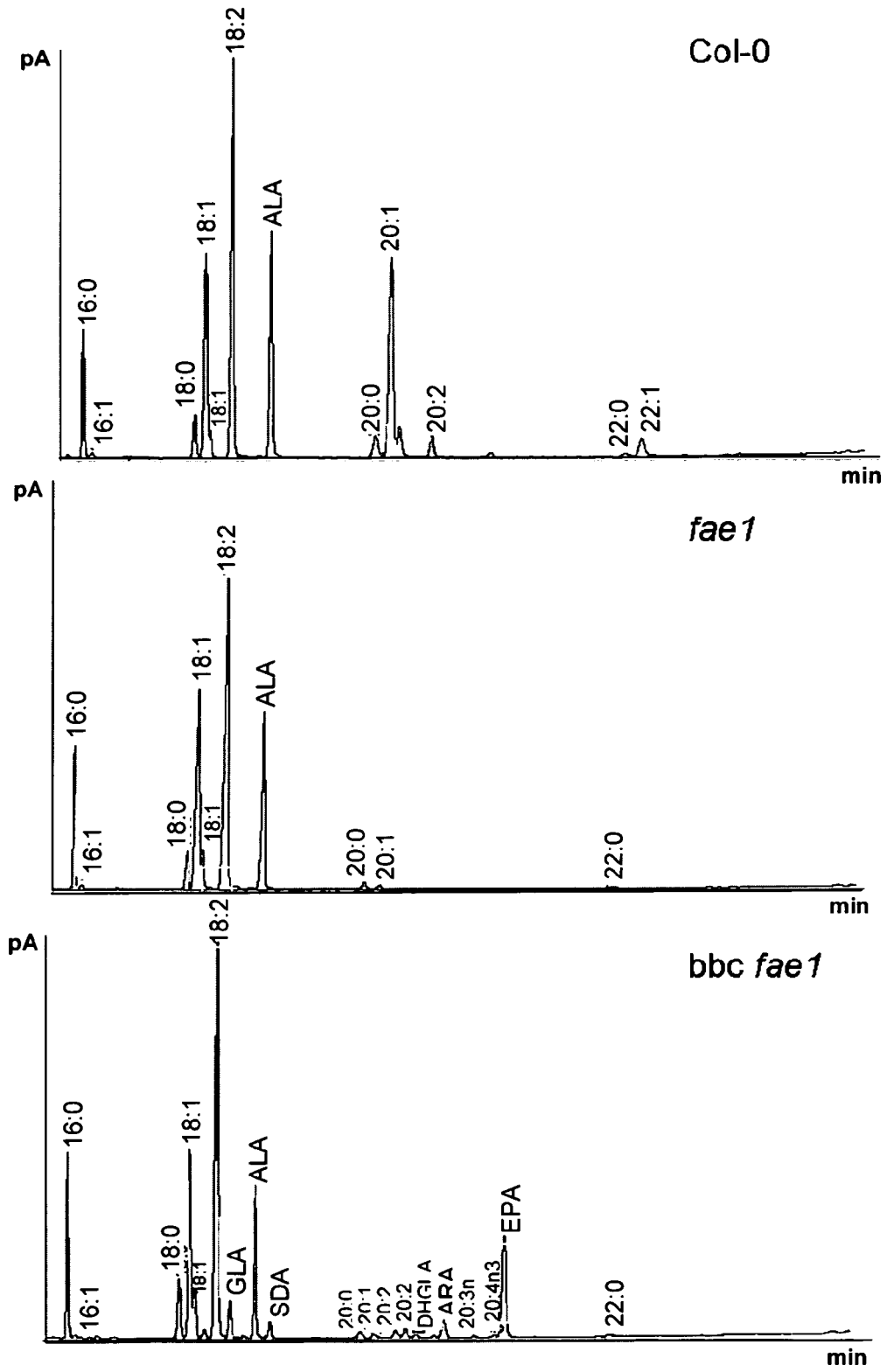


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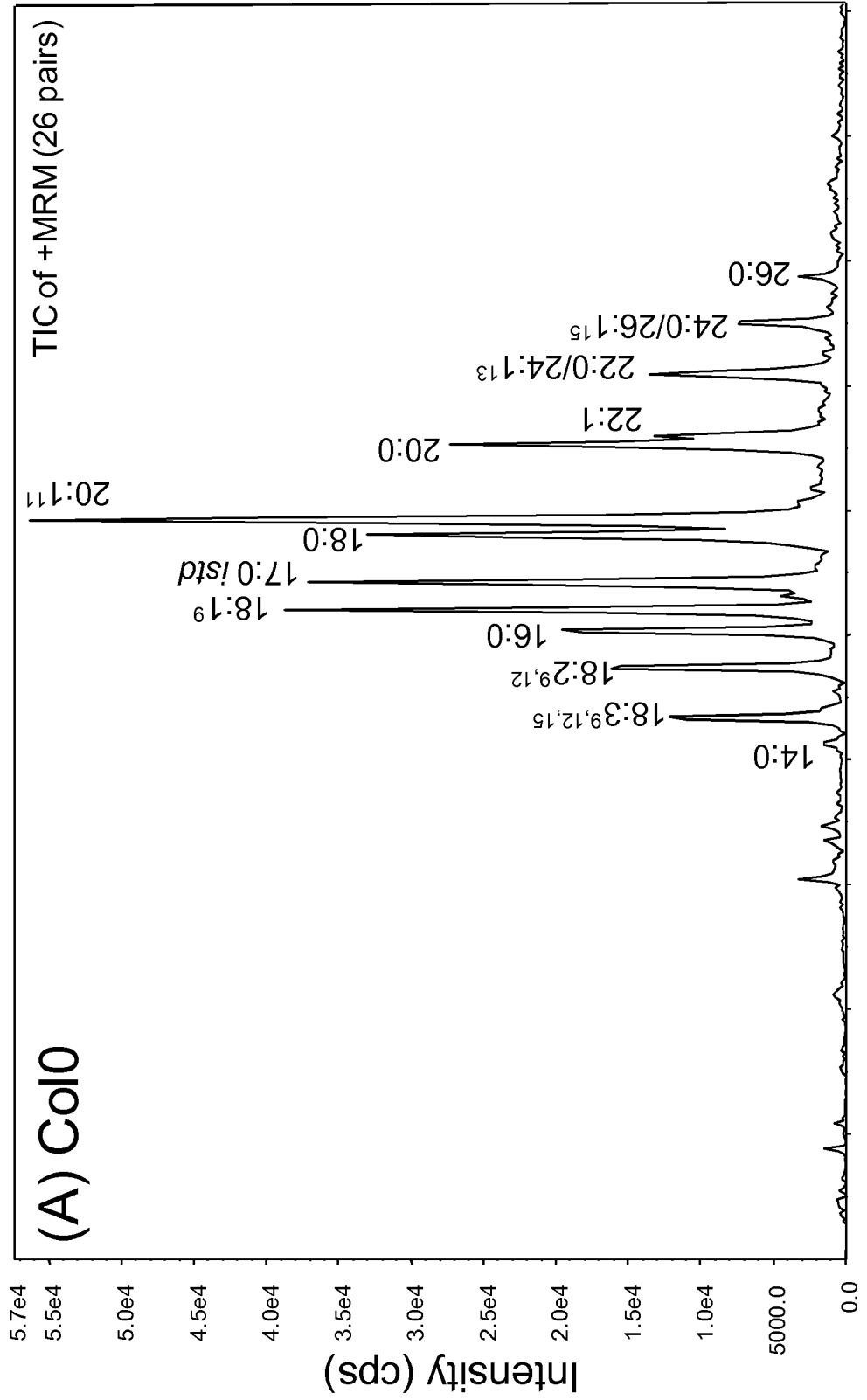


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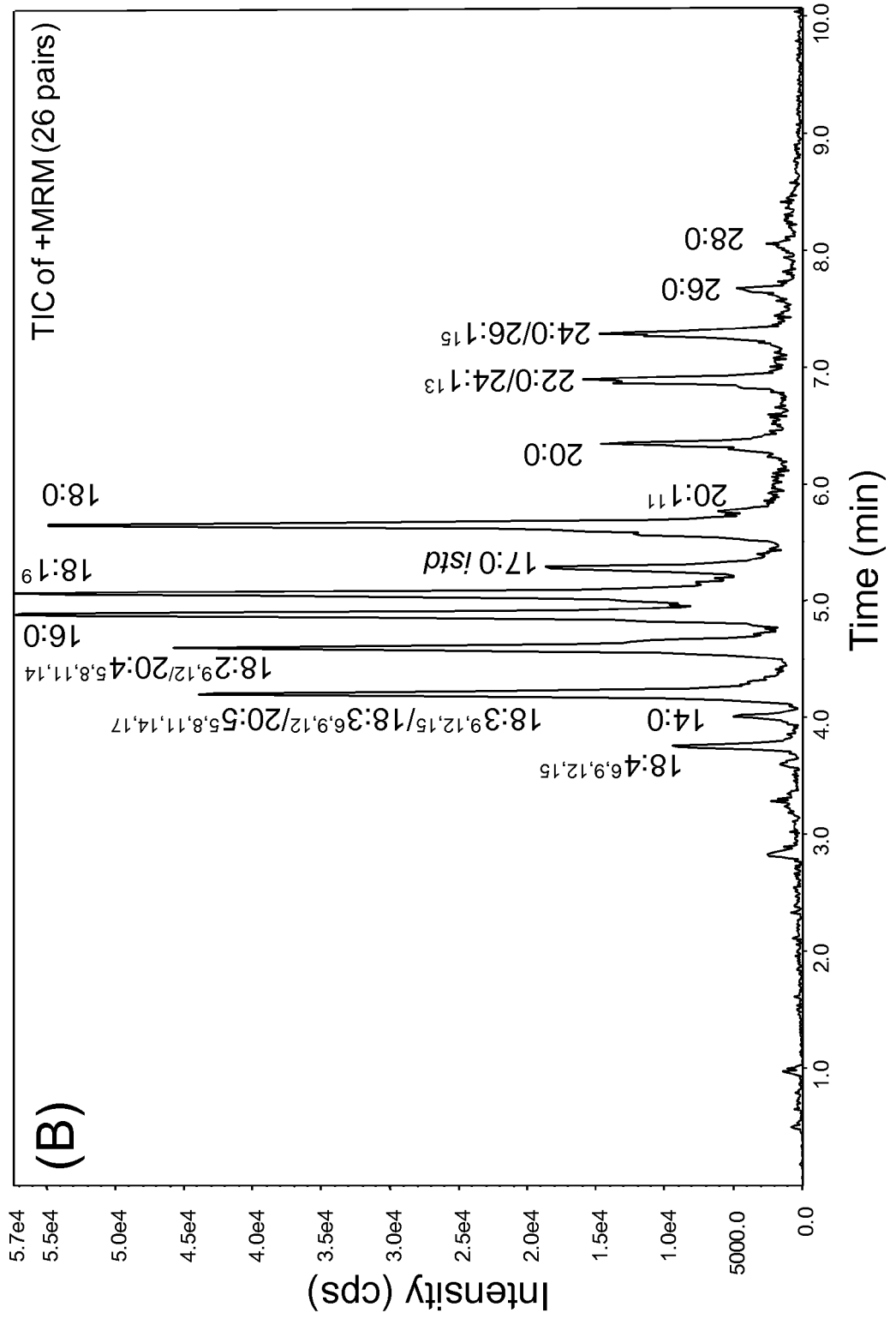


Fig 4 (continued):

Fig 5:

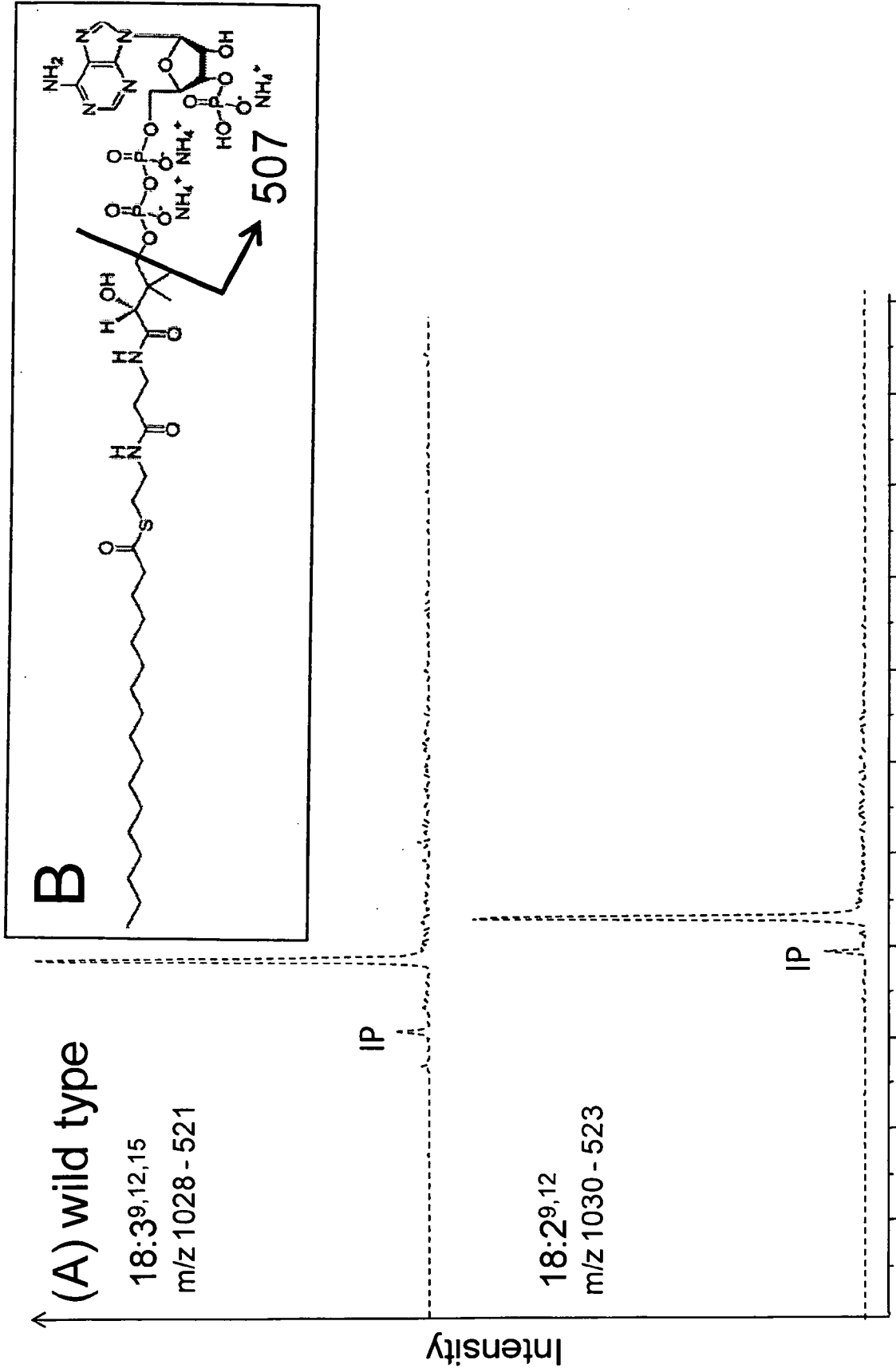


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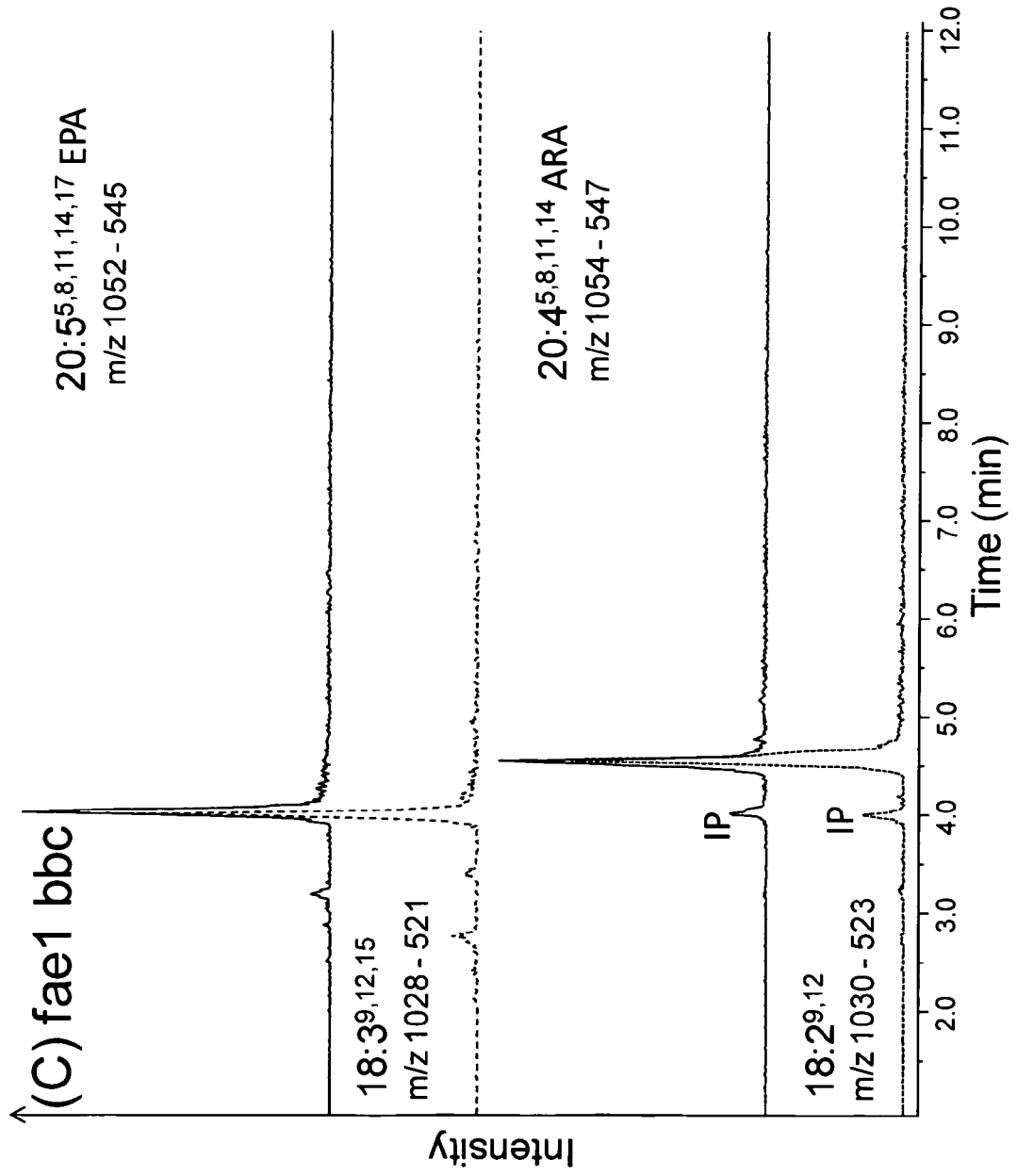


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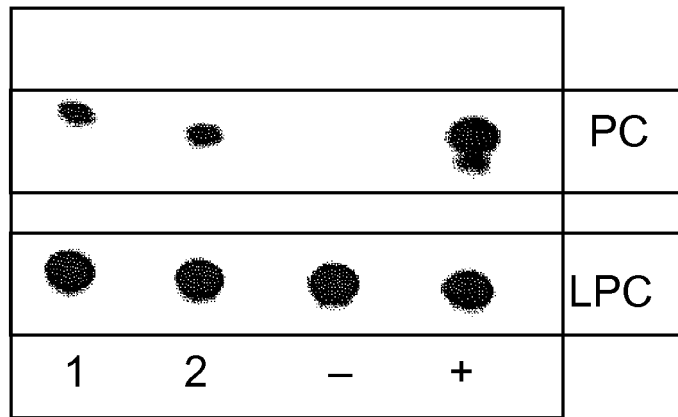
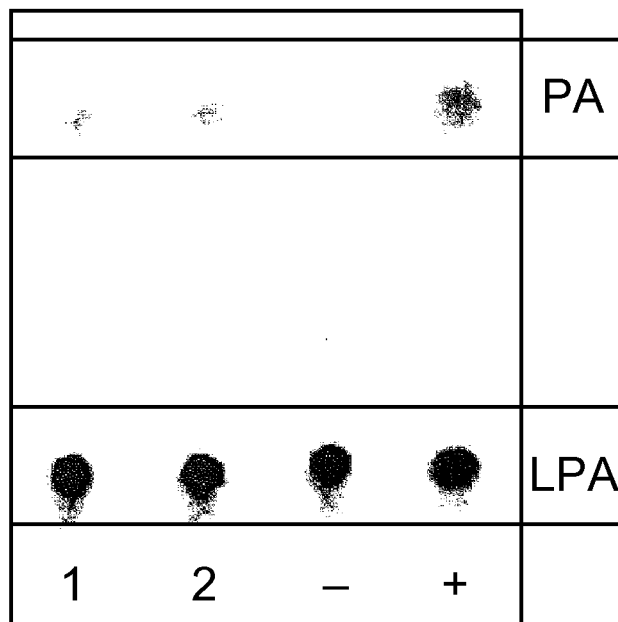


Fig 7:



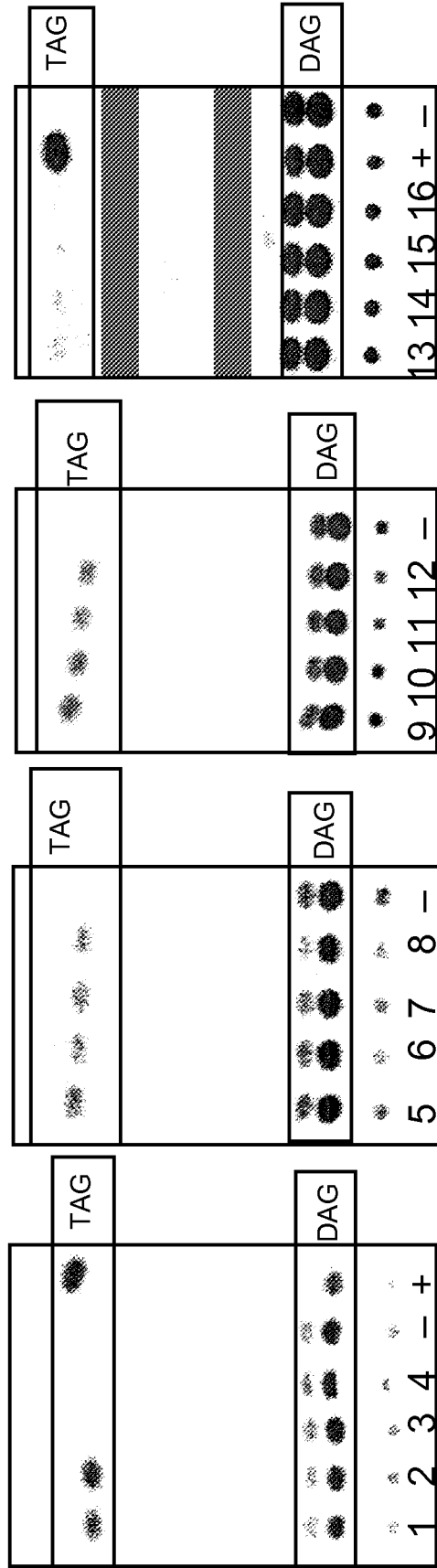


Fig 8

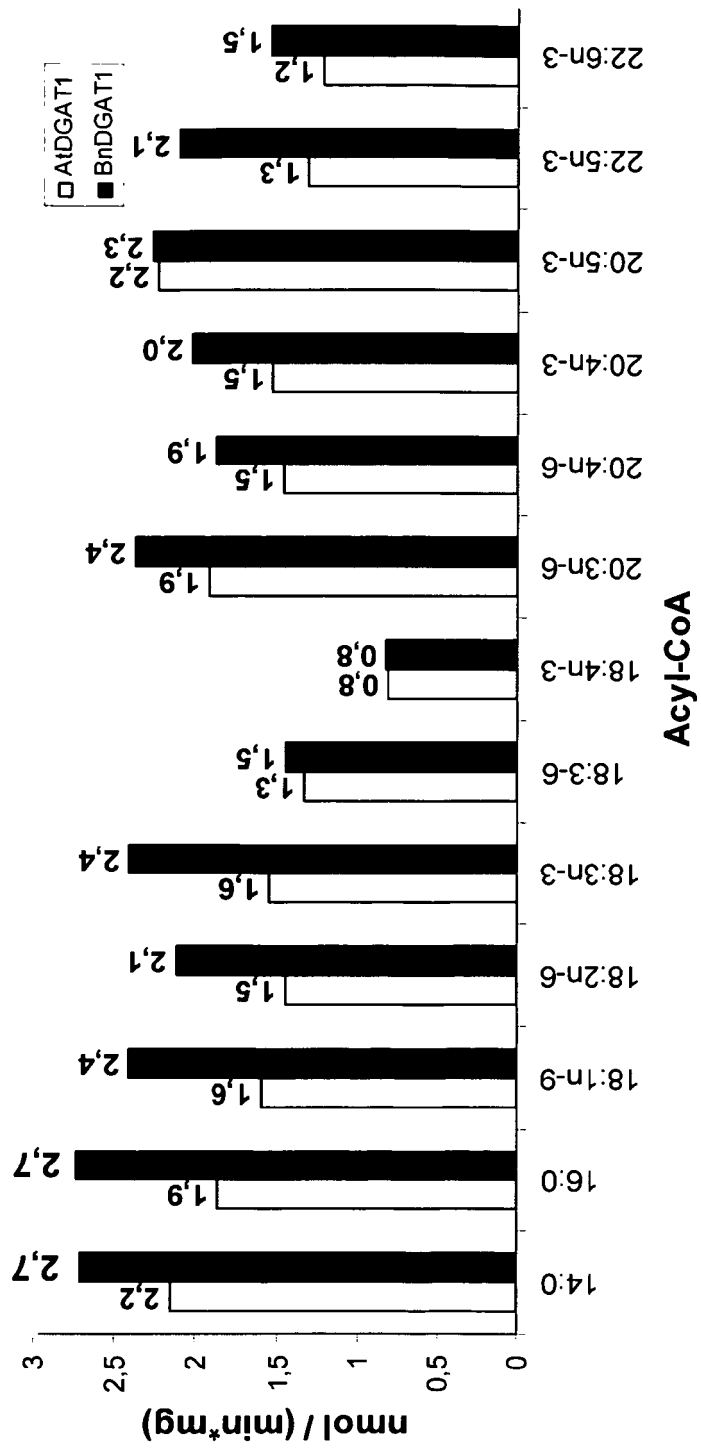


Fig9

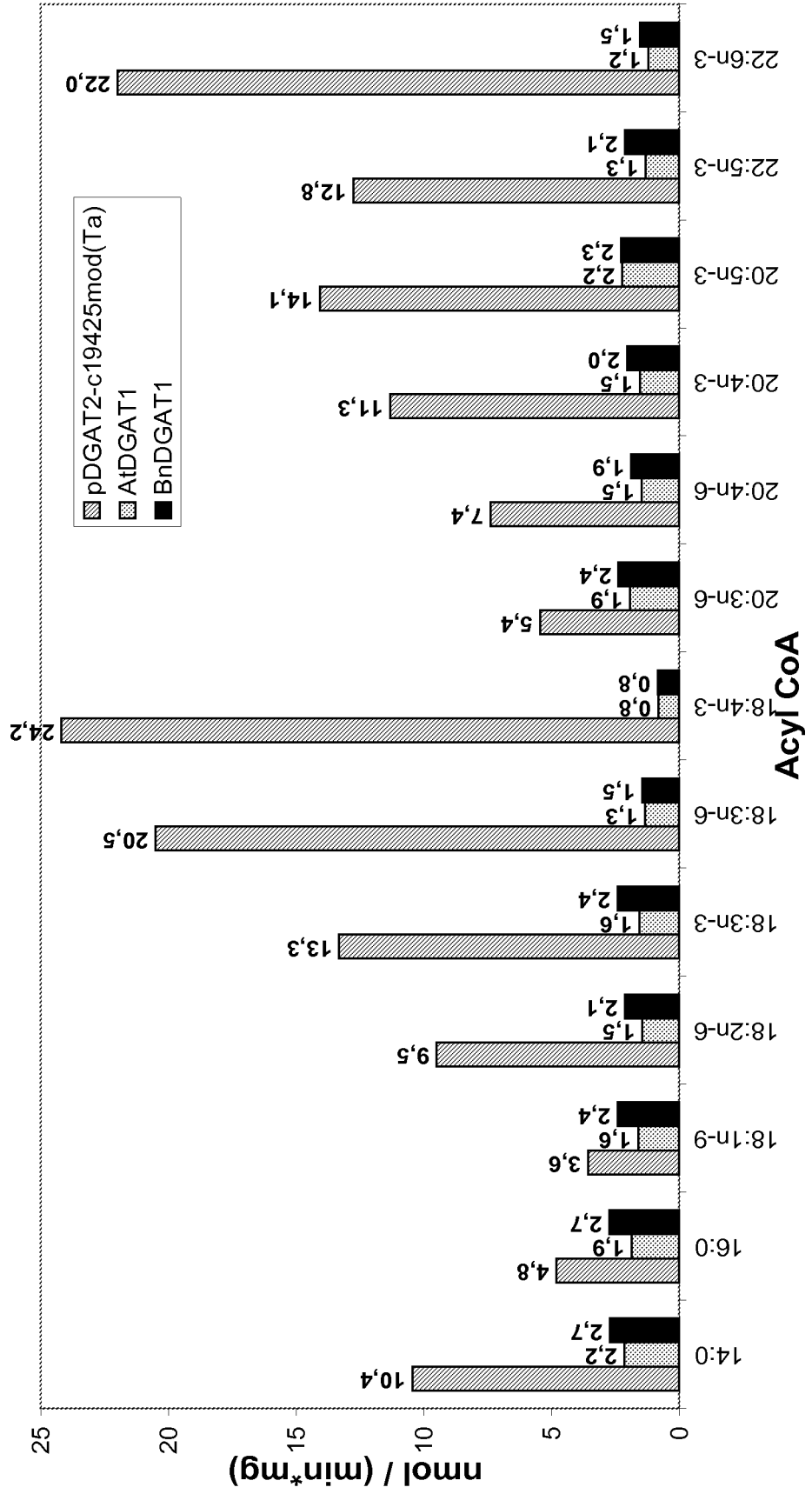


Fig 10

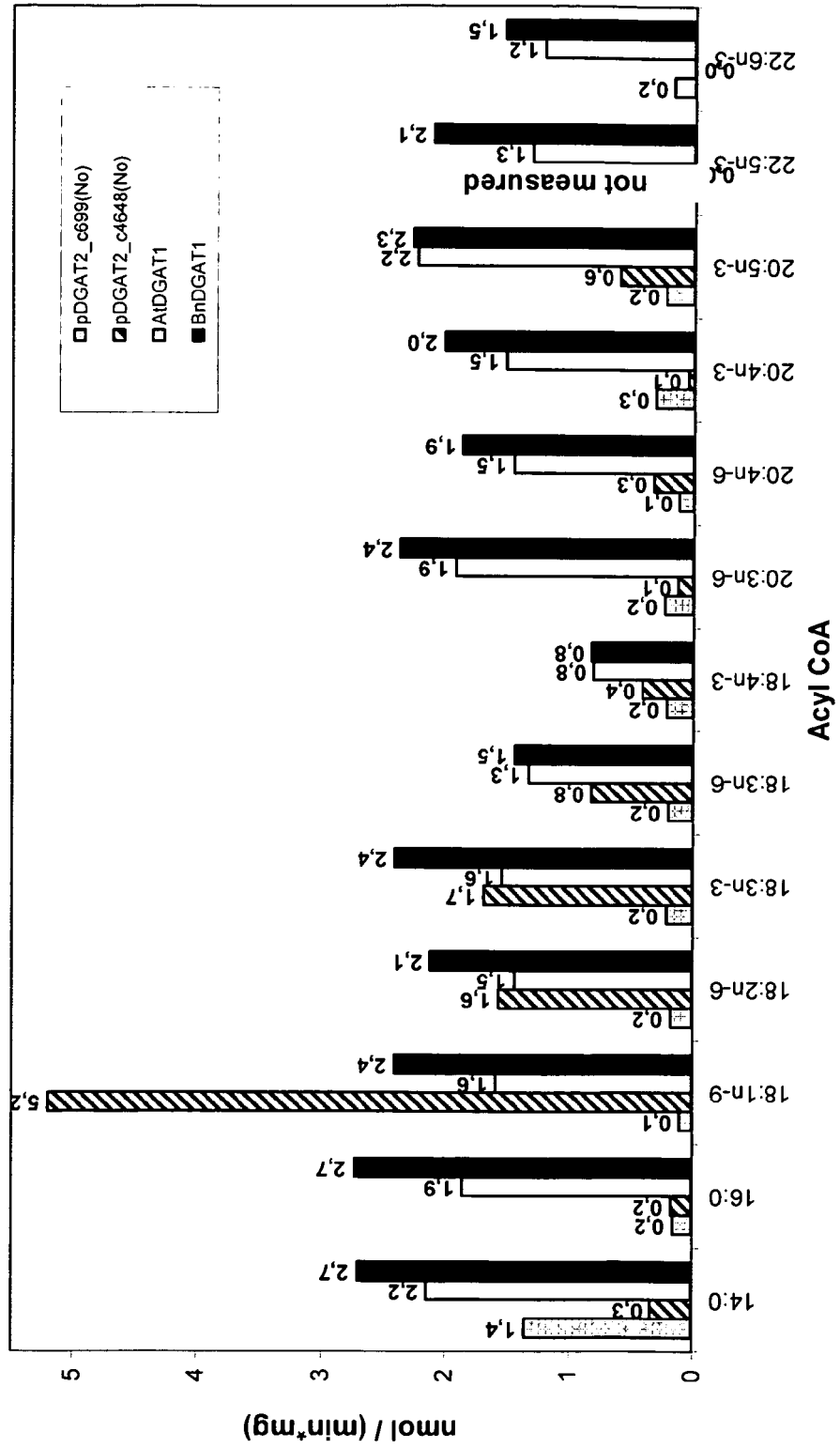


Fig 11

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