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(54) **FATTY ACID ELONGATION COMPONENTS AND USES THEREOF**

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C12N 9/02 (2006.01)
C12N 9/88 (2006.01)

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(2013.01); **C12P 7/6472** (2013.01)
USPC **800/281**; 800/298; 435/320.1; 435/419;
435/252.3; 435/69.1; 435/134; 536/23.2

(58) **Field of Classification Search**

None
See application file for complete search history.

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(57) **ABSTRACT**

The invention provides isolated nucleic acid molecules which encodes a novel fatty acid nECCR. The invention also provides recombinant expression vectors containing nECCR nucleic acid molecules, host cells into which the expression vectors have been introduced, and methods for large-scale production of long chain polyunsaturated fatty acids (LCPUFAs), e.g., ARA, EPA and DHA.

17 Claims, 12 Drawing Sheets

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Fig. 1

1 M A A A K Q Q Q S K G L G L K D L Y L I
1 atggctgctgctaacaacaacagtctaagggacttggacttaaggatctctacattata
1 taccgacgacgatttgttgggtcagattccctgaacctgaattcctagagatggaatat
21 T Y N A L C C L G W A Y V L A L G I P T
61 acttacaacgctttgtgttgccttggatgggcttacgttcttgccttggatccctacc
61 tgaatgttgcgaaacacaacagaaacctaccggaatgcaagaacgagaaccttagggatgg
41 F I A S V T S S I G T S S L V E S L K I
121 tttatcgcttctgtgacctcttctatcggaaacttctagccttgttgagtctcttaagatc
121 aatagcgaagacactggagaagatagccttgaagatcggaaactcagagaattctag
61 A G R S V Y A A T P Y T A G W S N E A T
181 gctggaagatctgtttacgctgctactccttacactgctggatgggtctaacgaggctact
181 cgaccttctagacaaatgcgacgatgaggaatgtgacgacctaccagattgctccgatga
81 P S L A T V L M Y V Q S A A V L E I V H
241 ctttctcttgcctaccgcttcttatgtacgttccagtctgctgctgttcttgagatcgttcc
241 ggaagagaacgatggcaagaatcatgcaagtcagacgacgacaagaactctagcaagtg
101 A A L G L V R S P V F V T T M Q V G S R
301 gctgctcttggacttgttagatctcctgttttcgtgacctatgcaagttggatcaaga
301 cgacgagaacctgaacaatctagaggacaaaagcactggatgatacgttcaacctagttct
121 I V A L H M L S T C P S A Q T Q W G A A
361 atcgttgcctctccatagctttctacttgccttctgctcaaactcaatggggagctgct
361 tagcaacgagaggtatacgaagatgaacaggaagacgagtttgagttaccctcgacga
141 L M I F S W A L V E V P R Y L F Y V A A
421 cttatgatcttctcttgggctcttgttgaagttcctcgttacctctctacgttggctgct
421 gaatactagaagagaacccgagaacaactcaaggagcaatggagaagatgcaacgacga
161 I V T G D A T K G T P Y P L F W L R Y S
481 atcgttactggatgctactaagggaactccttacccttggcttctggctcagatactct
481 tagcaatgacctacgatgattcccttgaggaatgggaacaagaccgagctctatgaga
181 L F A V L Y P T G I S G E L S V F L T S
541 ctttgcgctgttcttaccctactggaatctctggtgagttgtctgttttccctcactct
541 gaaaagcgacaagaatgggatgaccttagagacctcaacagacaaaaggagtgaaga
201 A K C D T F L S T L G E S N K S I M Y W
601 gctaagtgcgataccttcccttctaccctcggtgaaagcaacaagtctattatgtactgg
601 cgattcacgctatggaaggaaagatgggagccacttctggttgcagataatacatgacc
221 Y A M A F P I I Y A P G A L P M I F N M
661 tacgctatggctttccctattatctacgctcctgggtgctctccctatgatcttcaatag
661 atgcgataccgaaaggataatagatgcgaggaccacgagagggatactagaagttatac
241 V A N R K S A M K K R F A R P P P P R
721 gtggctaaccgtaagtctgctatgagaagagattcgcctagacctcctccacctcctaga
721 caccgattggcattcagacgatacttcttcttaagcgatctggaggagggtggaggatct
261 G L V W P V T E T K A N G E E V R S S T
781 ggacttgtttggcctgttactgagactaaggctaaccggtgaagaagttagatcttctacc
781 cctgaacaaaccggacaatgactctgattccgattgccacttcttcaatctagaagatgg
281 P V A K E I L A A A I G A V N P E L A E
841 cctgtggctaagagatccttgcctgctatcggagctgtaaacctgagcttgcctgag
841 ggacaccgatttctctaggaacgacgacgatagcctcgacaattgggactcgaacgactc
301 K V R N E K K W R F G Y Q K H L V N M V
901 aaagtgagaaacgagaagaagtgagattcggataccaaaagcacctcgtgaatatggtt
901 tttcactctttgctcttcttccacttaagcctatggtttctcgtggagcacttataccaa
321 E A Q C K S P E D A L K I A N A G L N K
961 gaggtcagtgtaagtctcctgaggatgctcttaagattgctaaccgggacttaacaag
961 ctccgagtcacattcagaggactcctacgagaattcctaacgattgctggcctgattgttc
341 A Y M T F Q F V S S D G S K T T T F A E
1021 gcttatatgaccttccagttcgtttcttctgatggatctaagactactacttctcgtgag
1021 cgaatatactggaaggtcaagcaagaagactacctagattctgatgatgaaagcagctc

Fig. 1 (Continued)

361 A M S S K S S D K F H T G F I K G E L A
1081 gctatgtctagcaagtctagcgataagttccacactggttttatcaagggtgaactcgct
1081 cgatacagatcgttcagatcgctattcaaggtgtgacccaaaatagttcccacttgagcga
381 P Q K E K K L E V G Y K G K Q I S G D E
1141 cctcaaaaagagaagaagctcgaagttggatacaagggaagcagatctctggtgatgag
1141 ggagtttttctcttcttcgagcttcaacccatgttccctttcgtctagagaccactactc
401 L K A Q V K E W V D Y G T I E P S A G E
1201 ctttaaggctcaagtgaagagtggttgactacggaactatcgagccttctgctggtgaa
1201 gaattccgagttcactttctcaccactgatgccttgatagctcggaaagcagaccactt
421 A I C S C V D N P G W I D L S D R Y F V
1261 gctatctgttcttgcggttgataaccctggatggatcgatctttctgacagatacttcggt
1261 cgatagacaagaacgcaactattgggacctacctagctagaaagactgtctatgaagcaa
441 L L G A G S A M G P F E V L M Q L G A N
1321 ctcttggagctggatctgctatgggacctttcgaggttttgatgcaacttgagtaac
1321 gaagaacctcgacctagacgataaccctggaaagctccaaaactacgttgaacctcgattg
461 V I G I D L D R P F I W Q R L I N R V M
1381 gttatcggaatcgacctcgacagaccttttatctggcagaggcttatcaacagagtgatg
1381 caatagccttagctggagctgtctggaaaatagaccgtctccgaatagttgtctcactac
481 N S S G S I T F P M S K E Q S K C A D E
1441 aactcttctggatctatcaccttccctatgtctaaagaacagagcaagtgcgctgatgag
1441 ttgagaagacctagatagtggaagggatacagatttctgtctcgttcacgcgactactc
501 K E L F A A S G C N L F T Q A P M I R D
1501 aaagagcttttctgctgcttctggatgcaaccttttctcactcaggctcctatgatcagagat
1501 tttctcgaaaagcgacgaagacctacgttggaaaagtgagtcgaggatactagtctcta
521 W L V D L Y P G K S F T V G S Y A Y L N
1561 tggctcgttgatctttaccctggaaagtcttctcactgtgggatcttacgcttaccttaac
1561 accgagcaactagaaatgggacctttcagaaaagtgacaccctagaatgccaatggaattg
541 G A L H V Q V S L A M D A I C R D L C D
1621 ggtgctctccacggttcaagtttctcttgctatggatgctatctgtcgtgatctctcgat
1621 ccacgagaggtgcaagttcaagagaacgatacctacgatagacagcactagagacgcta
561 K R K N T S L A Y L C T P T D L H L I P
1681 aagaggaagaacacttctcttgcttacctctgcactcctactgatcttcaccttatccct
1681 ttctccttctgtgaagagaacgaatggagacgtgaggatgactagaagtggaatagggga
581 K E A H D A A E A N Y K E F S K K P F C
1741 aaagaggctcacgacgctgctgaggctaaactacaaagagttcagcaagaaacctttctgt
1741 tttctccgagtgctgacgactccgattgatgtttctcaagtcgttctttggaaagaca
601 M F M K L F F G K K T L R K N V K K P V
1801 atgtttatgaagttgttcttccgaaagaaaaccctcagaaagaacgtgaagaagcctggt
1801 tacaataacttcaacaagaagcctttctttgggagcttttcttgcaacttcttcggacaa
621 S G V G G D F Y Y V N G I S V A Q G P N
1861 agcggagttggtggagatttctactacgtgaacggaatctctggttgcacaggacctaac
1861 tcgcctcaaccacctctaaagatgatgcacttgcccttagagacaacgagttcctggattg
641 Y A L A K R M Q H W R A V I A R S K G C
1921 tacgctcttgctaagagaatgcaacactggcgtgctgttatcgctagaagcaaggatgt
1921 atgcgagaacgattctcttacggttgaccgcacgacaatagcgatcttcggtccctaca
661 I V S S N I A P S T S T V S V T Q N R T
1981 atcgtgtctagcaatatcgctccttctacctctactgtttctgtgactcagaacagaact
1981 tagcacagatcgttatagcaggaagatggagatgacaagacactgagttctgtcttga
681 F A W A Y E G M P Y F K P Y E I F A P E
2041 ttcgcttgggcttacgaggaatgccttacttcaagccttacgagatcttcgctcctgag
2041 aagcgaacctgaatgctcccttacggaatgaagttcggaaatgctctagaagcagagactc
701 T S N S V M S A I L F N D L N N H K S I
2101 acttctaactctgtgatgagcgctatccttttcaacgatctcaacaaccacaagctatc
2101 tgaagattgagacactactcggataggaagttgctagagttgttggtgttcagatag

Fig. 1 (Continued)

721 A N P D V G I A N P N Q L F S F G A F H
2161 gctaaccctgatggttgaatcgctaaccctaaaccagcttttctctttcgggtgctttccac
2161 cgattgggactacaaccttagcgattgggtttggtcgaaaagagaaagccacgaaagggtg
741 G G T W R C A Y E I D S I G E A S V L L
2221 ggtggaacttggagatgtgcttacgagattgattctatcggagaggcttctgttcttctc
2221 ccaccttgaacctctacacgaatgctctaactaagatagcctctccgaagacaagaagag
761 Y F S R V A K P Y A I A F G G L G L A A
2281 tacttctctcgtggttgcataaaccttacgctatcgcttttcggaggacttggcttagctgct
2281 atgaagagagcacaacgatttggatgcatagcgaagcctcctgaaccagatcgacga
781 G A K W F G I V *
2341 ggtgctaagtgggttcggaattgtgtga
2341 ccacgattcaccaagccttaacacact

Fig. 2

1 M G L K D A Y L V L Y N S A C C A G W A
1 atgggacttaaggacgcttacctcgtgctttacaactctgcttgttggctggctgggct
1 taccctgaattcctgcgaatggagcacgaaatgttgagacgaacaacacgaccgacccga
21 Y V W Y A A C T T I L D K V A N Q S P F
61 tatgtttggtagcgtgttgtagctactatcctcgataaaggttgcaaacccagcaccttc
61 atacaaacctatgcgacgaacatgatgataggagctattccaacgtttggtagtgaaag
41 G D A S A Q V Y A H D D T A T M L T Y A
121 ggtgatgcttctgctcaagtttacgctcagcatgatactgctactatgcttacctacgct
121 ccactacgaagacgagttcaaagtcgagtgctactatgacgatgatacgaatggatgcca
61 Q S A A L L E I L H A A L G L V R S P V
181 caactgctgctccttgagatccttcacgctgctcctggacttggtagatctcctggt
181 gtttagacgacgagaggaactctaggaagtgcgacgagaaacctgaacaatctagaggaaa
81 M V T A M Q V M S R I V A L V A L V F S
241 atggtagcgcctatgcaagttatgtctaggatcgttgccttggctcctcgtgcttctct
241 taccactggcgatacgttcaatacagatcctagcaacgagaaacacgagagcacaagaga
101 S Q A Q T Q W G A G L M I I S W A S V E
301 tcacaagctcaaaactcaatggggagctggacttatgattatctcctgggcttcagttgaa
301 agtgctcgagtttgagttaccctcgacctgaataactaatagagaacctgaagtcactt
121 V P R Y A F Y V T A L L T G D A T K K T
361 gttcctcgttacgcttctacgcttactgctccttactggtgatgctactaagaaaacc
361 caaggagcaatgcgaaagatgcaatgacgagaggaatgaccactacgatgattcttttg
141 P F P L F W L R Y S L F A I L Y P T G I
421 cctttcctcttttctggcttctgcttactctcttctcttctccttaccctactggaatc
421 ggaaaggagaaaagaccgaagcaatgagagaaaagcgataggaaatgggatgaccttag
161 C G E L T V F L A A S K D Q A F V D K F
481 tgtggagagcttactgttttctcctcgtgcttctcaaggatcaagcttctcgtggataagttc
481 acacctctcgaatgacaaaaggagcgacgaagattcctagttcgaaaagcacctattcaag
181 G P L S V T L Y S I V L P I V Y F F G S
541 aagcctccttctggttactctctactctatcgttctcctatcgtgtacttctcggatct
541 cctggagaaagacaatgagagatgagatagcaagaggatagcacatgaagaagcctaga
201 P F M I M N M V A N R K S A F K K R F A
601 ccttttatgattatgaatgatggggctaacagaaagtctgcttcaagaagagattcgca
601 ggaaaataactaatacttataaccaccgattgtccttccagacgaaagttcttctcctaagcgt
221 K P P P P A R G L C W P V D A K G Q R S
661 aagcctccacctcctgctagaggactttgtggcctggtgatgctaagggacagagatct
661 ttccgaggtggaggacgatctcctgaaacaaccggacaactacgattccctgtctctaga
241 S T N V N K T I I A A A V G A V N E Q K
721 tctaccaacgtgaacaagacgattattgctgctgctggtggagctgtaaacgagcaaaag
721 agatggttgcaactgttctgctaataacgacgacgacaacctcgacaattgctcgttttc
261 A E A I R S C K A W R F Q Y V K H L R A
781 gctgaggtatcagatcttgtaagcctggagattccagtagcttaagcaccttagagct
781 cgactccgatagtctagaacattccgaacctctaaggtcatgcaattcgtggaatctcga
281 M V E E Q C Q T P E S A L K I A Q A G L
841 atggttaggaacaatgtcaaactcctgagctcgtctcctaagatcgcctcaagctggactt
841 taccactcctgttacagtttgaggactcagacgagaattctagcgagttcgacctgaa
301 D S A Y D I F E F V A P D G S A T F R
901 gattctgcttacgatatcttcgagttcggttgcctcctgatggatctgctactcttcag
901 ctaagacgaatgctatagaagctcaagcaacgaggactacctagacgatgatgaaagtc
321 E A M A A K N T E Q F F T H V I K G E G
961 gaagctatggctgctaagaacactgagcagttcttactcacgttatcaaggagagggga
961 cttcgataaccgacgattcttggtagctcgtcaagaagtgagtgcaatagttccctctccct
341 N K L T K E L E I P Y K G G I L K G D A
1021 aacaagcttaccaaagagcttgagatcccttacaaggggtggaatccttaagggtgact
1021 ttggtcgaatggtttctcgaactctaggaatgttccaccttaggaattccactacga
361 L K K Q V Q S W A D Y G T I E P S A G A
1081 ctaagagcaggttcagcttgggctgattacggaactatcagccttctcgtggtgct
1081 gaattctcgtccaagtcagaacctgactaatgccttgatagctcggagacgaccacga

Fig. 2 (Continued)

381 A I V K C I E H P E W L D I S N R Y F V
1141 gctatcggttaagtgtatcgagcaccctgagtggttgatcagcaacagatacttcggt
1141 cgatagcaattcacatagctcggtggactcaccgaactatagtcggttgctatgaagcaa
401 L L G A G S A M G P L L V L M A L G A N
1201 cttctggagctggatctgctatgggacctcttctgttcttatggctctcgagctaac
1201 gaagaacctcgacctagacgataccctggagaagaacaagaataccgagagcctcgattg
421 V I A V D L D R P N I W K R L I D I A R
1261 gttatcgctgtggatcttgatagacctaatatctggaagcgtcttatcgatcgcgtaga
1261 caatagcgacacctagaactatctggattatagacctcgcagaatagctatagcgatct
441 Q S S G T I T F P M K M D P S K C K N D
1321 caatcttctggaacgatcaccttccctatgaagatggaccctagcaagtgaagaacgac
1321 gttagaagaccttgctagtgaagggatacttctacctgggatcggttcacattcttgctg
461 E E M F A Q A G C N L F T D T P M I R D
1381 gaggaaatggtcgcctcaggctggatgtaaccttttcaccgacacccctatgatcagagat
1381 ctctttacaagcgagtcggacctacattggaaaagtggtgtggtgggatactagtcteta
481 W L M N V Y P G K S L T V G C Y A Y L D
1441 tggttgatgaacgtttaccctggaaagtctcttactgttgatgctacgcttaccttgat
1441 accaactacttgcaaatgggacctttcagagaatgacaacctacgatgccaatggaacta
501 G A L H V Q V S L A M D A I C R D L S E
1501 ggtgctctccacgttcaagtttctcttgctatggatgctatctgcccgtgatctttctgag
1501 ccacgagaggtgcaagttcaagagaacgataacctacgatagacggcactagaagactc
521 K R K N T S L A Y L C T P T D L H L I P
1561 aagaggaagaacacttctcttgcttacctctgcaactcctactgatcttcaacttgatccct
1561 ttctccttcttggaagagaacgaatggagacgtgaggatgactagaagtgaaactagga
541 K E A H D A M K A N Y K S Y S G K L Y C
1621 aaagaggtcacgatgctatgaaggctaactacaagtcttactccgaaagctttactgt
1621 tttctccgagtgctacgatacttccgattgatggtcagaatgaggcctttcgaaatgaca
561 M M M N L L S G G K F L R Q N S K K P V
1681 atgatgatgaaccttctcagcggaggaaagttccttagacagaactctaagaagcctggt
1681 tactactacttgaagagtcgctccttcaaggaatctgctctgagattcttcggacaa
581 S G K G G E Y Y L V N G I S V A Q G P N
1741 tctgaaaggggtgagagtagtactaccttgtaacggaatctctgttgctcaagacctaac
1741 agacctttccacctctcatgatggaacacttgcccttagagacaacgagttcctggattg
601 Y A L A K R M Q H W R A I T A R N K G C
1801 tacgctcttgctaagagaatgcaacactggcgtgctatcactgctagaaacaagggatgt
1801 atgcgagaacgattctcttactggtgacccgacgatagtgacgatctttgttccctaca
621 I V S S N I A P S T S T V S V V H N R T
1861 atcgtgctagcaatattgctccttctacctctaccgtttctgttggttcacaacagaact
1861 tagcacagatcggtataacgaggaagatggagatggcaagacaacaagtgtgtcttga
641 F A W A Y E G M P Y F E P F E I F A P E
1921 ttccgttgggttacgagggaaatgccttacttcgagcctttcgagatcttctgctcctgag
1921 aagcgaacccgaatgctccttaccggaatgaagctcggaaagctctagaagcggagactc
661 T S N A V M S A L L F Y D L N D S G S W
1981 acttctaacgctgttatgtctgctctcctcttctacgatctcaacgattctggatcttgg
1981 tgaagattgcaacaatacagacgagagagaagatgctagagttgctaagacctagaacc
681 A T P N T S L G N P N Q L F S H G S F H
2041 gctactcctaacttctctcgaaacccctaaccagcttttctctcaccgatctttccac
2041 cgatgaggttggaagagagcctttgggattggtcgaagagagtgcttagaagagtg
701 G G V W R C A Y E V D S I G E S S V L L
2101 ggtggagtttgagagatgtgcttaccgaggttgactctatcggagaatcttctgtgcttctc
2101 ccacctcaaacctctacacgaatgctccaactgagatagcctcttagaagacacgaagag
721 Y F G R V A K P Y M V A A G A V A A G
2161 tacttggaaagagtggttaaaccttatatggttgctgctggtgcccgtggccgcagctggt
2161 atgaagccttctcaccgatttggaaatataccaacgacgaccacggcaccggcgtcgacca
741 A A Y V Y A V *
2221 gcagcctacgtttacgctgtgta
2221 cgctggatgcaaatgacacact

Fig. 3

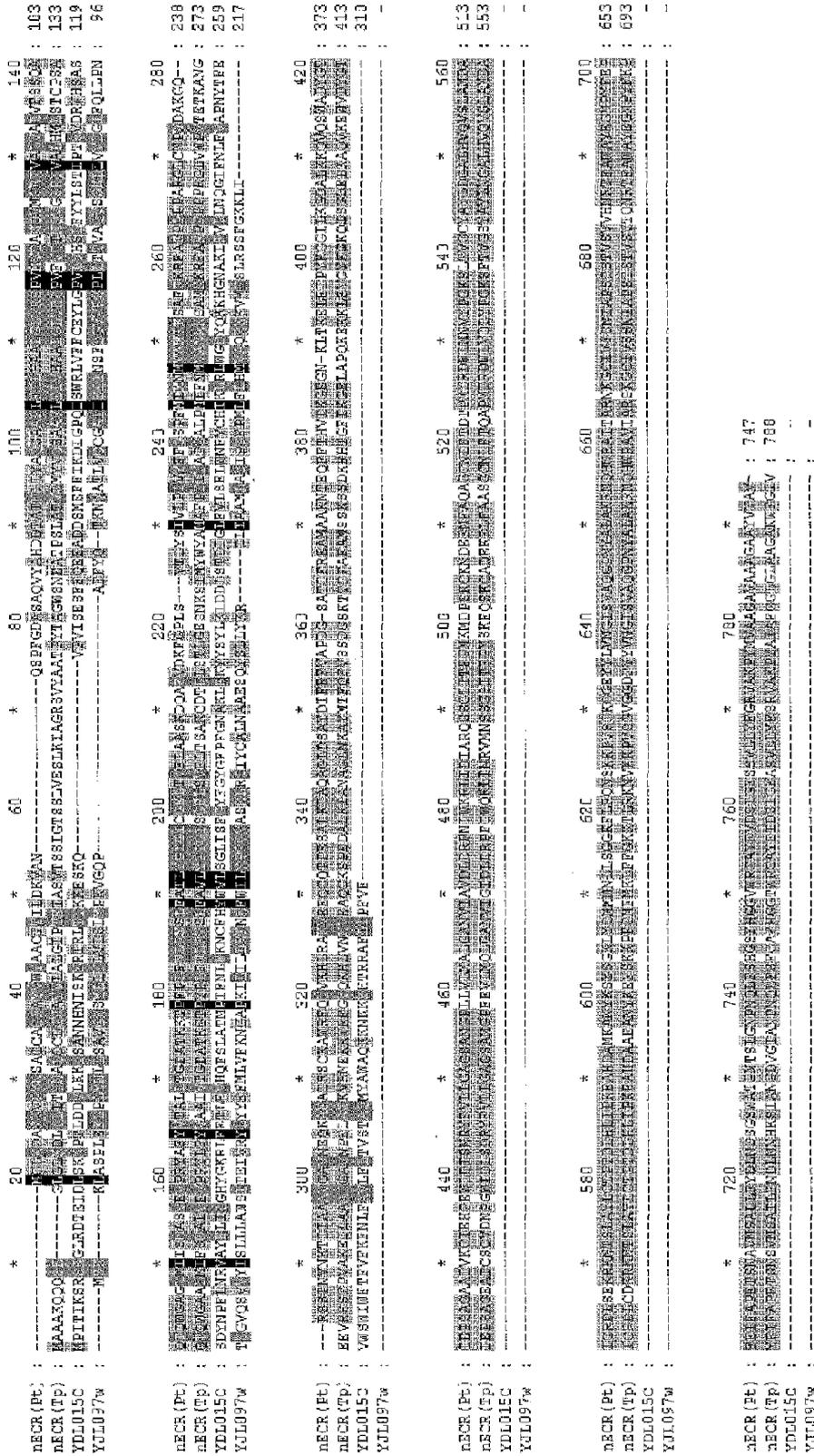


Fig. 4

YDL015C	nECR(P)	nECR(Tp)	YJL097W
nECR(P)	8	8	12
nECR(Tp)		65	25
YJL097W			27

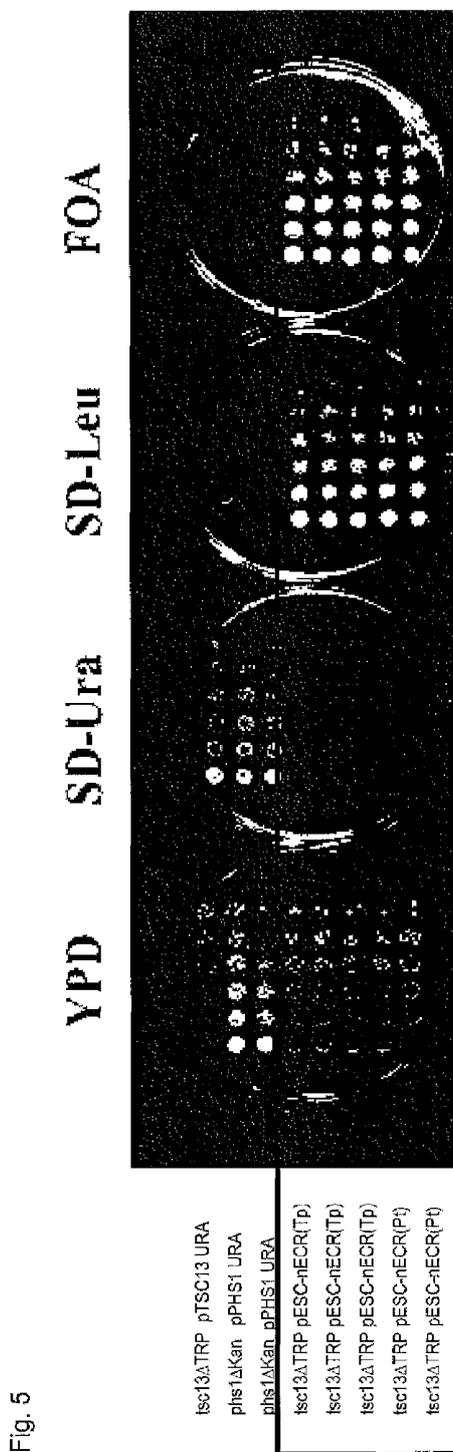


Fig. 6

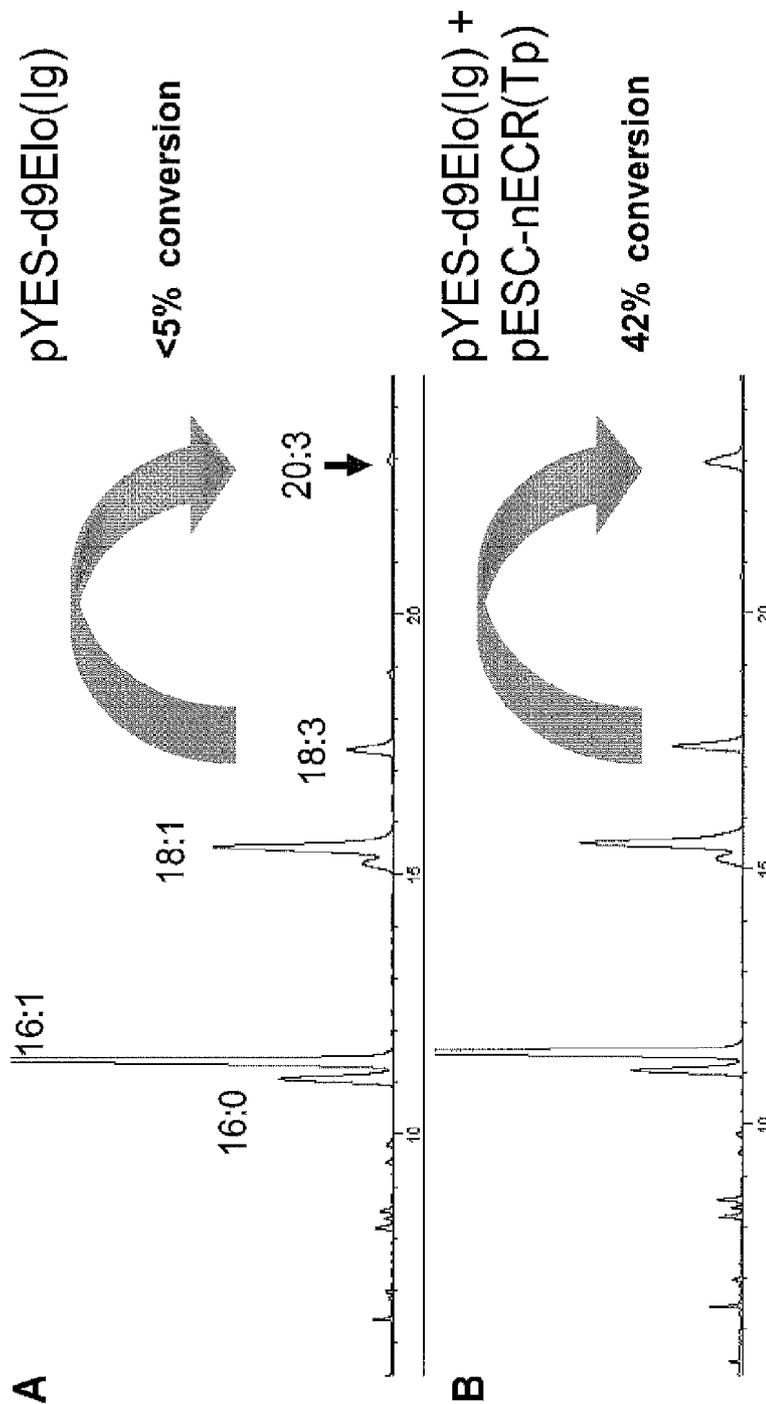
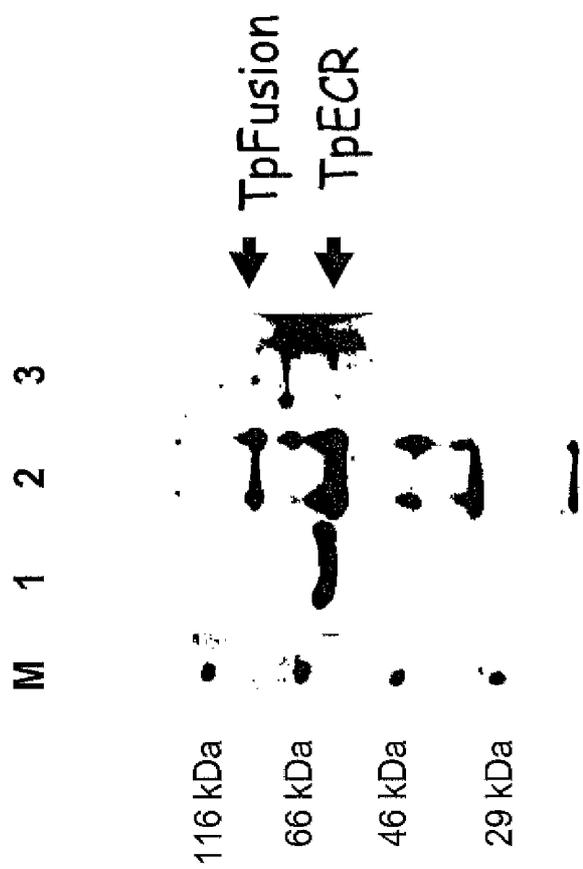


Fig. 7



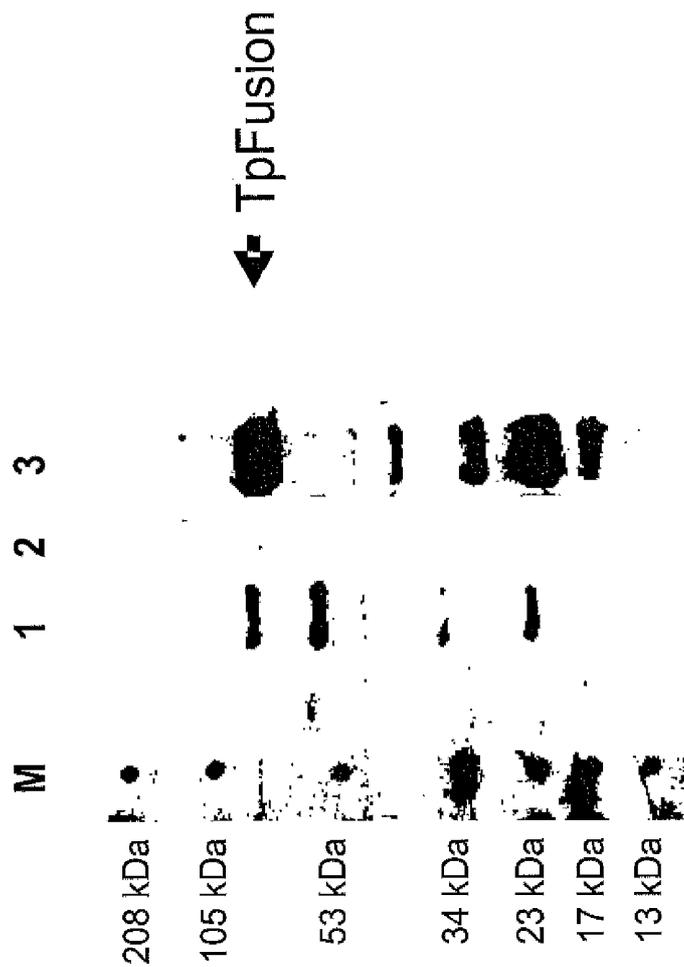


Fig. 8

Fig. 9



FATTY ACID ELONGATION COMPONENTS AND USES THEREOF

RELATED APPLICATIONS

This application is a national stage application (under 35 U.S.C. §371) of PCT/EP2010/056936, filed May 20, 2010 which claims benefit of European Application No. 09162204.3 filed Jun. 8, 2009.

SUBMISSION OF SEQUENCE LISTING

The Sequence Listing associated with this application is filed in electronic format via EFS-Web and hereby incorporated by reference into the specification in its entirety. The name of the text file containing the Sequence Listing is Sequence_Listing_17418__00078 U.S. The size of the text file is 72 KB and the text file was created on Dec. 5, 2011.

The invention in principle pertains to the field of recombinant manufacture of fatty acids. It provides nucleic acid molecules which encode novel fatty acid dehydratase/enoyl-CoA reductase (nECR) family members. The invention also provides recombinant expression vectors containing nECR nucleic acid molecules, host cells into which the expression vectors have been introduced, and methods for large-scale production of long chain polyunsaturated fatty acids (LCPUFAs), e.g. ARA, EPA and DHA.

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

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

Long chain polyunsaturated fatty acids (LCPUFAs) such as docosahexaenoic acid (DHA, 22:6(4,7,10,13,16,19)) are essential components of cell membranes of various tissues and organelles in mammals (nerve, retina, brain and immune cells). For example, over 30% of fatty acids in brain phospholipid are 22:6 (n-3) and 20:4 (n-6) (Crawford, M. A., et al, (1997) *Am. J. Clin. Nutr.* 66:1032S-1041S). In retina, DHA accounts for more than 60% of the total fatty acids in the rod outer segment, the photosensitive part of the photoreceptor

cell (Giusto, N. M., et al. (2000) *Prog. Lipid Res.* 39:315-391). Clinical studies have shown that DHA is essential for the growth and development of the brain in infants, and for maintenance of normal brain function in adults (Martinetz, M. (1992) *J. Pediatr.* 120: S129-S138). DHA also has significant effects on photoreceptor function involved in the signal transduction process, rhodopsin activation, and rod and cone development (Giusto, N. M., et al. (2000) *Prog. Lipid Res.* 39:315-391). In addition, some positive effects of DHA were also found on diseases such as hypertension, arthritis, atherosclerosis, depression, thrombosis and cancers (Horrocks, L. A. and Yeo, Y. K. (1999) *Pharmacol. Res.* 40:211-215). Therefore, appropriate dietary supply of the fatty acid is important for human health. Because such fatty acids cannot be efficiently synthesized by infants, young children and senior citizens, it is particularly important for these individuals to adequately intake these fatty acids from the diet (Spector, A. A. (1999) *Lipids* 34:S1-S3).

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

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

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

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

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

- a) a nucleic acid sequence having a nucleotide sequence as shown in SEQ ID NOs: 1 or 3;

- b) a nucleic acid sequence encoding a polypeptide having an amino acid sequence as shown in SEQ ID NOs: 2 or 4;
- c) a nucleic acid sequence being at least 50% identical to the nucleic acid sequence of a) or b), wherein said nucleic acid sequence encodes a polypeptide having dehydratase/enoyl-CoA reductase (nECR) activity;
- d) a nucleic acid sequence encoding a polypeptide having nECR activity and having an amino acid sequence which is at least 50% identical to the amino acid sequence of any one of a) to c); and
- e) a nucleic acid sequence which is capable of hybridizing under stringent conditions to any one of a) to d), wherein said nucleic acid sequence encodes a polypeptide having nECR activity.

The term "polynucleotide" as used in accordance with the present invention relates to a polynucleotide comprising a nucleic acid sequence which encodes a polypeptide having dehydratase/enoyl-CoA reductase (nECR) activity. Preferably, the polypeptide encoded by the polynucleotide of the present invention having nECR activity upon expression in a plant shall be capable of increasing the amount of PUFA and, in particular, LCPUFA in, e.g., seed oils or the entire plant or parts thereof. Such an increase is, preferably, statistically significant when compared to a LCPUFA producing transgenic control plant which expresses the minimal set of desaturases and elongases required for LCPUFA synthesis but does not express the polynucleotide of the present invention. Whether an increase is significant can be determined by statistical tests well known in the art including, e.g., Student's t-test. More preferably, the increase is an increase of the amount of triglycerides containing LCPUFA of at least 5%, at least 10%, at least 15%, at least 20% or at least 30% compared to said control. Preferably, the LCPUFA referred to before is a polyunsaturated fatty acid having a C-20, C-22 or C24 fatty acid body, more preferably, ARA, EPA or DHA. Suitable assays for measuring the activities mentioned before are described in the accompanying Examples.

The term "nECR activity" or "dehydratase/enoyl-CoA reductase activity" as used herein refers to the combined activity of a enoyl-CoA reductase and a dehydratase, i.e. the enzyme having the combined activity shall be capable of removing a hydroxyl group from 3-hydroxy-acyl-CoA and reducing the formed double bond as part of the elongation process for fatty acids. Fatty acid elongation is catalyzed in four steps, represented by four enzymes: KCS (keto-acyl-CoA-synthase), KCR (keto-acyl-CoA-reductase), DH (dehydratase) and ECR (enoyl-CoA-reductase). In the first step a fatty acid-CoA ester is condensed with malonyl-CoA producing a keto-acyl-CoA intermediate, which is elongated by two carbon atoms, and CO₂. The keto-group of the intermediate is then reduced by the KCR to a hydroxyl-group. In the next step the DH cleaves of the hydroxyl-group (H₂O is produced), forming a acyl-2-en-CoA ester (delta-2-enoyl-CoA). In the final step the double bond at position 2, 3 is reduced by the ECR forming the elongated acyl-CoA ester (Buchanan, Grisse, Jones (2000) Biochemistry & Molecular biology of plants, American Society of Plant Physiologists). In the studies underlying this invention, a natural occurring fusion of DH and ECR with superior catalytic activities and specificities towards LCPUFA has been provided.

More preferably, polynucleotides having a nucleic acid sequence as shown in SEQ ID NOs: 1 or 3 encoding polypeptides having amino acid sequences as shown in SEQ ID NOs: 2 or 4 or variants thereof, preferably, exhibit nECR activity.

A polynucleotide encoding a polypeptide having a nECR activity as specified above has been obtained in accordance

with the present invention, preferably, from *Thalassiosira pseudonana* or *Phaeodactylum tricorutum*. However, orthologs, paralogs or other homologs may be identified from other species. Preferably, they are obtained from plants such as algae, for example *Isochrysis*, *Mantoniella*, *Ostreococcus* or *Cryptocodinium*, algae/diatoms such as *Phaeodactylum*, *Thalassiosira* or *Thraustochytrium*, mosses such as *Phycomitrella* or *Ceratodon*, or higher plants such as the Primulaceae such as *Aleuritia*, *Calendula stellata*, *Osteospermum spinescens* or *Osteospermum hyoseroides*, microorganisms such as fungi, such as *Aspergillus*, *Phytophthora*, *Entomophthora*, *Mucor* or *Mortierella*, bacteria such as *Shewanella*, yeasts or animals. Preferred animals are nematodes such as *Caenorhabditis*, insects or vertebrates. Among the vertebrates, the nucleic acid molecules may, preferably, be derived from Euteleostomi, Actinopterygii; Neopterygii; Teleostei; Euteleostei, Protacanthopterygii, Salmoniformes; Salmonidae or Oncorhynchus, more preferably, from the order of the Salmoniformes, most preferably, the family of the Salmonidae, such as the genus *Salmo*, for example from the genera and species *Oncorhynchus mykiss*, *Trutta trutta* or *Salmo trutta fario*. Moreover, the nucleic acid molecules may be obtained from the diatoms such as the genera *Thalassiosira* or *Phaeodactylum*.

Thus, the term "polynucleotide" as used in accordance with the present invention further encompasses variants of the aforementioned specific polynucleotides representing orthologs, paralogs or other homologs of the polynucleotide of the present invention. Moreover, variants of the polynucleotide of the present invention also include artificially generated muteins, Said muteins include, e.g., enzymes which are generated by mutagenesis techniques and which exhibit improved or altered substrate specificity, or codon optimized polynucleotides. The polynucleotide variants, preferably, comprise a nucleic acid sequence characterized in that the sequence can be derived from the aforementioned specific nucleic acid sequences shown in any one of SEQ ID NOs: 1 or 3 or by a polynucleotide encoding a polypeptide having an amino acid sequence as shown in any one of SEQ ID NOs: 2 or 4 by at least one nucleotide substitution, addition and/or deletion, whereby the variant nucleic acid sequence shall still encode a polypeptide having a nECR activity as specified above. Variants also encompass polynucleotides comprising a nucleic acid sequence which is capable of hybridizing to the aforementioned specific nucleic acid sequences, preferably, under stringent hybridization conditions. These stringent conditions are known to the skilled worker and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y. (1989), 6,3 ,1-6.3.6, A preferred example for stringent hybridization conditions are hybridization conditions in 6 x sodium chloride/sodium citrate (=SSC) at approximately 45° C., followed by one or more wash steps in 0.2 xSSC, 0 ,1% SDS at 50 to 65° C. The skilled worker knows that these hybridization conditions differ depending on the type of nucleic acid and, for example when organic solvents are present, with regard to the temperature and concentration of the buffer. For example, under "standard hybridization conditions" the temperature differs depending on the type of nucleic acid between 42° C. and 58° C. in aqueous buffer with a concentration of 0.1 to 5 xSSC (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 ,1xSSC and 20° C. to 45° C., preferably between 30° C. , and 45° C. The hybridization conditions for DNA :RNA hybrids are, preferably, 0,1xSSC and 30° C. to 55° C. preferably between 45°

and 55 °C. The abovementioned hybridization temperatures are determined for example for a nucleic acid with approximately 100 bp (=base pairs) in length and a G =C content of 50m% in the absence of formamide. The skilled worker knows how to determine the hybridization conditions required by referring to textbooks such as the textbook mentioned above, or the following textbooks: Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989; Hames and Higgins (Ed.) 1985, "Nucleic Acids Hybridization: A Practical Approach", IRL Press at Oxford University Press, Oxford; Brown (Ed.) 1991, "Essential Molecular Biology: A Practical Approach", IRL Press at Oxford University Press, Oxford. Alternatively, polynucleotide variants are obtainable by PCR-based techniques such as mixed oligonucleotide primer-based amplification of DNA, i.e. using degenerated primers against conserved domains of the polypeptides of the present invention. Conserved domains of the polypeptide of the present invention may be identified by a sequence comparison of the nucleic acid sequences of the polynucleotides or the amino acid sequences of the polypeptides of the present invention. Oligonucleotides suitable as PCR primers as well as suitable PCR conditions are described in the accompanying Examples. As a template, DNA or cDNA from bacteria, fungi, plants or animals may be used. Further, variants include polynucleotides comprising nucleic acid sequences which are at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the nucleic acid sequences shown in any one of SEQ 1 D NOs: 1 or 3, preferably, encoding polypeptides retaining a ECR activity as specified above. Moreover, also encompassed are polynucleotides which comprise nucleic acid sequences encoding a polypeptide having an amino acid sequences which are at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequences shown in any one of SEQ ID NOs: 2 or 4, wherein the polypeptide, preferably, retains nECR activity as specified above. The percent identity values are, preferably, calculated over the entire amino acid or nucleic acid sequence region. A series of programs based on a variety of algorithms is available to the skilled worker for comparing different sequences. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch algorithm (Needleman 1970, J. Mol. Biol. (48):444-453) which has been incorporated into the needle program in the EMBOSS software package (EMBOSS: The European Molecular Biology Open

Software Suite, Rice,P., Longden,I., and Bleasby,A, Trends in Genetics 1.6(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 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 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. 215:403-40). BLAST using nECR 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 nECR sequences of the invention. BLAST using nECR 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 nECR 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

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 nECR activity as specified above. Accordingly, the polypeptide may comprise or consist of the domains of the polypeptide of the present invention conferring the said biological activity. A fragment as meant herein, preferably, comprises at least 50, at least 100, at least 250 or at least 500 consecutive nucleotides of any one of the aforementioned nucleic acid sequences or encodes an amino acid sequence comprising at least 20, at least 30, at least 50, at least 80, at least 100 or at least 150 consecutive amino acids of any one of the aforementioned amino acid sequences.

The variant polynucleotides or fragments referred to above, preferably, encode polypeptides retaining nECR 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 nECR activity exhibited by any of the polypeptide shown in any one of SEQ ID NOs: 2 or 4. The activity may be tested as described in the accompanying Examples.

The polynucleotides of the present invention either essentially consist of the aforementioned nucleic acid sequences or comprise the aforementioned nucleic acid sequences. Thus, they may contain further nucleic acid sequences as well.

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

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

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

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

In the studies underlying the present invention, advantageously, polynucleotides where identified encoding dehydratases from *Thalassiosira pseudonana* and *Phaeodactylum tricornumtum*. In particular, the *Thalassiosira pseudonana* and *Phaeodactylum* dehydratase/enoyl-CoA-reductase nECR have been identified [nECR(Tp) and nECR(Pt)]. Each of these nECR are capable of removing a hydroxyl group from 3-hydroxy-acyl-CoA and reducing the formed double bond as part of the elongation process for fatty acids. For example, the expression of the nECR(Tp) and nECR(Pt) in a

Saccharomyces cerevisiae mutant not been able to elongate fatty acids has been found to restore the elongation process. The polynucleotides of the present invention are particularly suitable for the recombinant manufacture of LCPUFAs and, in particular, ARA, EPA and/or DHA.

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

The term "expression control sequence" as used herein refers to a nucleic acid sequence which is capable of governing, i.e. initiating and controlling, transcription of a nucleic acid sequence of interest, in the present case the nucleic acid 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. a abscisic-acid-inducible promoter) or WO 93/21334 (i.e. a ethanol- or cyclohexenol-inducible promoter). Further suitable plant promoters are the promoter of cytosolic FBPase or the ST-LSI promoter from potato (Stockhaus 1989, EMBO J. 8, 2445), the phosphoribosyl-pyrophosphate amidotransferase promoter from Glycine max (Genbank accession No. U87999) or the node-specific promoter described in EP 0 249 676 A1. Particularly preferred are promoters which enable the expression in tissues which are involved in the biosynthesis of fatty acids. Also particularly preferred are seed-specific promoters such as the USP promoter in accordance with the practice, but also other promoters such as the LeB4, DC3, phaseolin or napin promoters. Further especially preferred promoters are seed-specific promoters which can be used for monocotyledonous or dicotyledonous plants and which are described in U.S. Pat. No. 5,608,152 (napin promoter from oilseed rape), WO 98/45461 (oleosin promoter from *Arabidopsis*, U.S. Pat. No. 5,504,200 (phaseolin promoter from *Phaseolus vulgaris*), WO 91/13980 (Bce4 promoter from *Brassica*), by Baumlein et al., Plant J., 2, 2, 1992:233-239 (LeB4 promoter from a legume), these promoters being suitable for dicots. The following promoters are suitable for monocots: Ipt-2 or Ipt-1 promoter from barley (WO 95/15389 and WO 95/23230), hordein promoter from barley and other promoters which are suitable and which are described in WO 99/16890. In principle, it is possible to use all natural promoters together with their regulatory sequences, such as those mentioned above, for the novel process. Likewise, it is possible and advantageous to use synthetic promoters, either

additionally or alone, especially when they mediate a seed-specific expression, such as, for example, as described in WO 99/16890. In a particular embodiment, seed-specific promoters are utilized to enhance the production of the desired PUFA or LCPUFA.

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

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

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

The term "vector", preferably, encompasses phage, plasmid, viral vectors as well as artificial chromosomes, such as bacterial or yeast artificial chromosomes. Moreover, the term also relates to targeting constructs which allow for random or site-directed integration of the targeting construct into genomic DNA. Such target constructs, preferably, comprise DNA of sufficient length for either 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 co-precipitation, DEAE-dextran-mediated transfection, lipofection, natural competence, carbon-based clusters, chemically mediated transfer, electroporation or particle

bombardment. Suitable methods for the transformation or transfection of host cells, including plant cells, can be found in Sambrook et al. (*Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989) and other laboratory manuals, such as *Methods in Molecular Biology*, 1995, Vol. 44, *Agrobacterium protocols*, Ed.: Gartland and Davey, Humana Press, Totowa, New Jersey. Alternatively, a plasmid vector may be introduced by heat shock or electroporation techniques. Should the vector be a virus, it may be packaged in vitro using an appropriate packaging cell line prior to application to host cells.

Preferably, the vector referred to herein is suitable as a cloning vector, i.e. replicable in microbial systems. Such vectors ensure efficient cloning in bacteria and, preferably, yeasts or fungi and make possible the stable transformation of plants. Those which must be mentioned are, in particular, various binary and co-integrated vector systems which are suitable for the T-DNA-mediated transformation. Such vector systems are, as a rule, characterized in that they contain at least the vir genes, which are required for the *Agrobacterium*-mediated transformation, and the sequences which delimit the T-DNA (T-DNA border). These vector systems, preferably, also comprise further cis-regulatory regions such as promoters and terminators and/or selection markers with which suitable transformed host cells or organisms can be identified. While co-integrated vector systems have vir genes and T-DNA sequences arranged on the same vector, binary systems are based on at least two vectors, one of which bears vir genes, but no T-DNA, while a second one bears T-DNA, but no vir gene. As a consequence, the last-mentioned vectors are relatively small, easy to manipulate and can be replicated both in *E. coli* and in *Agrobacterium*. These binary vectors include vectors from the pBIB-HYG, pPZP, pBecks, pGreen series. Preferably used in accordance with the invention are Bin19, pBI101, pBinAR, pGPTV and pCAMBIA. An overview of binary vectors and their use can be found in Hellens et al, *Trends in Plant Science* (2000) 5, 446-451. Furthermore, by using appropriate cloning vectors, the polynucleotides can be introduced into host cells or organisms such as plants or animals and, thus, be used in the transformation of plants, such as those which are published, and cited, in: *Plant Molecular Biology and Biotechnology* (CRC Press, Boca Raton, Florida), chapter 6/7, pp. 71-119 (1993); F. F. White, *Vectors for Gene Transfer in Higher Plants*; in: *Transgenic Plants*, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, 15-38; B. Jenes et al., *Techniques for Gene Transfer*, in: *Transgenic Plants*, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press (1993), 128-143; Potrykus 1991, *Annu. Rev. Plant Physiol. Plant Molec. Biol.* 42, 205-225.

More preferably, the vector of the present invention is an expression vector. In such an expression vector, i.e. a vector which comprises the polynucleotide of the invention having the nucleic acid sequence operatively linked to an expression control sequence (also called "expression cassette") allowing expression in prokaryotic or eukaryotic cells or isolated fractions thereof. Suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (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, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.), where glutathione S-transferase (GST), maltose E-binding protein and protein A, respectively, are fused with the recombinant target protein. Examples of suitable induc-

ible nonfusion *E. coli* expression vectors are, inter alia, pTrc (Amann 1988, Gene 69:301-315) and pET 11d (Studier 1990, Methods in Enzymology 185, 60-89). The target gene expression of the pTrc vector is based on the transcription from a hybrid trp-lac fusion promoter by host RNA polymerase. The target gene expression from the pET 11d vector is based on the transcription of a T7-gn10-lac fusion promoter, which is mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is provided by the host strains BL21 (DE3) or HMS174 (DE3) from a resident λ -prophage which harbors a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter. The skilled worker is familiar with other vectors which are suitable in prokaryotic organisms; these vectors are, for example, in *E. coli*, pLG338, pACYC184, the pBR series such as pBR322, the pUC series such as pUC18 or pUC19, the M113mp series, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III113-B1, 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, Calif.). 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, YEep6, YEep13 or pEM-BLYe23. As an alternative, the polynucleotides of the present invention can be also expressed in insect cells using baculovirus expression vectors. Baculovirus vectors which are available for the expression of proteins in cultured insect cells (for example Sf9 cells) comprise the pAc series (Smith 1983, Mol. Cell Biol. 3:2156-2165) and the pVL series (Lucklow 1989, Virology 170:31-39).

The polynucleotide of the present invention can be expressed in single-cell plant cells (such as algae, see Falciatore 1999, Marine Biotechnology 1 (3):239-251 and the references cited therein, and plant cells from higher plants (for example *Spermatophytes*, such as arable crops) by using plant expression vectors. Examples of plant expression vectors comprise those which are described in detail in: Becker 1992, Plant Mol. Biol. 20:1195-1197; Bevan 1984, Nucl. Acids Res. 12:8711-8721; Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, p. 15-38. A plant expression cassette, preferably, comprises regulatory sequences which are capable of controlling the gene expression in plant cells and which are functionally linked so that each sequence can fulfill its function, such as transcriptional termination, for example polyadenylation signals. Preferred polyadenylation signals are those which are derived from *Agrobacterium tumefaciens* T-DNA, such as the gene 3 of the Ti plasmid pTiACH5, which is known as octopine synthase (Gielen 1984, EMBO J. 3, 835) or functional equivalents of these, but all other terminators which are functionally active in plants are also suitable. Since plant gene expression is very often not limited to transcriptional levels, a plant expression cassette preferably comprises other functionally linked sequences such as translation enhancers, for example the overdrive sequence, which comprises the 5'-un-

translated 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 U.S. Pat. No. 5,352,605 and WO 84/02913) or plant promoters such as the promoter of the Rubisco small subunit, which is described in U.S. Pat. No. 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 Kermodé 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 (U.S. Pat. No. 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 (U.S. Pat. No. 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* (U.S. Pat. No. 5,504,200), the Bce4 promoter from *Brassica* (WO 91/13980) or the legumin B4 promoter (LeB4; Baeumlein 1992, Plant Journal, 2 (2):233-9), and promoters which bring about the seed-specific expression in monocotyledonous plants such as maize, barley, wheat, rye, rice and the like. Suitable promoters to be taken into consideration are the lpt2 or lpt1 gene promoter from barley (WO 95/15389 and WO 95/23230) or those which are described in WO 99/16890 (promoters from the barley hordein gene, the rice glutelin gene, the rice oryzin gene, the rice prolamin gene, the wheat gliadin gene, wheat glutelin gene, the maize zein gene, the oat glutelin gene, the sorghum kasirin gene, the rye secalin gene). Likewise, especially suitable are promoters which bring about the plastid-specific expression since plastids are the compartment in which the precursors and some end products of lipid biosynthesis are synthesized. Suitable promoters such as the viral RNA-polymerase promoter, are described in WO 95/16783 and WO 97/06250, and the clpP promoter from *Arabidopsis*, described in WO 99/46394.

The abovementioned vectors are only a small overview of vectors to be used in accordance with the present invention. Further vectors are known to the skilled worker and are

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

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

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

Preferably, said host cell is a plant cell and, more preferably, a plant cell obtained from an oilseed crop. More preferably, said oilseed crop is selected from the group consisting of flax (*Linum* sp.), rapeseed (*Brassica* sp.), soybean (*Glycine* and *Soja* sp.), sunflower (*Helianthus* sp.), cotton (*Gossypium* sp.), corn (*Zea mays*), olive (*Olea* sp.), safflower (*Carthamus* sp.), cocoa (*Theobroma 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.

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

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

Generally, the controlling steps in the production of LCPUFAs, i.e., the long chain unsaturated fatty acid biosynthetic pathway, are catalyzed by membrane-associated fatty acid elongase complexes. Plants and most other eukaryotic organisms have specialized elongase system for the extension of fatty acids beyond C18 atoms. These elongase reactions have several important features in common with the fatty acid synthase complex (FAS). However, the elongase complex is different from the FAS complex as the complex is localized in the cytosol and are membrane bound, ACP is not involved and the elongase 3-keto-acyl-CoA-synthase catalyzes the condensation of malonyl-CoA with an acyl primer. The elongase complex consists of four components with different catalytic functions, the keto-acyl-CoA-synthase (KCS, condensation reaction of malonyl-CoA to acyl-CoA, creation of a 2 C atom longer keto-acyl-CoA fatty acid), the keto-acyl-CoA-reductase (KCR, reduction of the 3-keto group to a 3-hydroxy-group), the dehydratase (DH, dehydration results in a delta-2-enoyl-acyl-CoA fatty acid) and the enoyl-CoA-reductase (ECR, reduction of the double bond at position 2, release from the complex). For the production of LCPUFAs including ARA, EPA and/or DHA the elongation reaction could be essential. Higher plants do not have the necessary enzyme set to produce LCPUFAs (4 or more double bonds, 20 or more C atoms). Therefore the catalytic activities have to be conferred to the plants or plant cells. One critical step in the process of elongation is the dehydration and reduction reaction. The polynucleotides of the present invention surprisingly catalyze the dehydration and reduction activity by one enzyme. By delivering this nECR increased levels of PUFAs and LCPUFAs are produced.

However, it will be understood that dependent on the host cell, further, enzymatic activities may be conferred to the host cells, e.g., by recombinant technologies. Accordingly, the present invention, preferably, envisages a host cell which in addition to the polynucleotide of the present invention comprises polynucleotides encoding such desaturases and/or elongases as required depending on the selected host cell. Preferred desaturases and/or elongases which shall be present in the host cell are at least one enzyme selected from the group consisting of: 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 tricorutum* (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 tricorutum* (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 d5d6EIo(Om)2 from *Oncorhynchus mykiss* (WO2005012316), d5d6EIo(Ta) from *Thraustochytrium aureum* (WO2005012316) and d5d6EIo(Tc) from *Thraustochytrium* sp. (WO2005012316), the d5-elongases d5EIo(At) from *Arabidopsis thaliana* (WO2005012316), d5EIo(At)2 from *Arabidopsis thaliana* (WO2005012316), d5EIo

(Ci) from *Ciona intestinalis* (WO2005012316), d5EIo(OI) from *Ostreococcus lucimarinus* (WO2008040787), d5EIo(Ot) from *Ostreococcus tauri* (WO2005012316), d5EIo(Tp) from *Thalassiosira pseudonana* (WO2005012316) and d5EIo(XI) from *Xenopus laevis* (WO2005012316), the d6-elongases d6EIo(OI) from *Ostreococcus lucimarinus* (WO2008040787), d6EIo(Ot) from *Ostreococcus tauri* (WO2005012316), d6EIo(Pi) from *Phytophthora infestans* (WO2003064638), d6EIo(Pir) from *Pythium irregulare* (WO2009016208), d6EIo(Pp) from *Physcomitrella patens* (WO2001059128), d6EIo(Ps) from *Phytophthora sojae* (WO2006100241), d6EIo(Ps)2 from *Phytophthora sojae* (WO2006100241), d6EIo(Ps)3 from *Phytophthora sojae* (WO2006100241), d6EIo(Pt) from *Phaeodactylum tricorutum* (WO2005012316), d6EIo(Tc) from *Thraustochytrium* sp. (WO2005012316) and d6EIo(Tp) from *Thalassiosira pseudonana* (WO2005012316), the d9-elongases d9EIo(Ig) from *Isochrysis galbana* (WO2002077213), d9EIo(Pm) from *Perkinsus marinus* (WO2007093776) and d9EIo(Ro) from *Rhizopus oryzae* (WO2009016208). Particularly, if the manufacture of ARA is envisaged in higher plants, the enzymes recited in Table 3, below (i.e. additionally a d6-desaturase, d6-elongase, d5-elongase, d5-desaturase, d12-desaturase, and d6-elongase) or enzymes having essentially the same activity may be combined in a host cell. If the manufacture of EPA is envisaged in higher plants, the enzymes recited in Table 4, below (i.e. additionally a d6-desaturase, d6-elongase, d5-desaturase, d12-desaturase, d6-elongase, omega 3-desaturase and d15-desaturase), or enzymes having essentially the same activity may be combined in a host cell. If the manufacture of DHA is envisaged in higher plants, the enzymes recited in Table 5, below (i.e. additionally a d6-desaturase, d6-elongase, d5-desaturase, d12-desaturase, d6-elongase, omega 3-desaturase, d15-desaturase, d5-elongase, and d4-desaturase), or enzymes having essentially the same activity may be combined in a host cell.

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

The present invention furthermore pertains to a method for the manufacture of a polypeptide encoded by a polynucleotide of any the present invention comprising

a) cultivating the host cell of the invention under conditions which allow for the production of the said polypeptide; and

b) obtaining the polypeptide from the host cell of step a).

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

tioned method, the polypeptides produced thereby may become posttranslationally modified or processed otherwise.

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

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

Encompassed by the present invention is, furthermore, an antibody which specifically recognizes the polypeptide of the invention.

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

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

Preferably, the non-human transgenic organism is a plant, plant part, or plant seed. Preferred plants to be used for introducing the polynucleotide or the vector of the invention are plants which are capable of synthesizing fatty acids, such as all dicotyledonous or monocotyledonous plants, algae or mosses. It is to be understood that host cells derived from a plant may also be used for producing a plant according to the present invention. Preferred plants are selected from the

group of the plant families Adelotheceaceae, Anacardiaceae, Asteraceae, Apiaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Caricaceae, Cannabaceae, Convolvulaceae, Chenopodiaceae, Cryptocodiniaceae, 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: Adelotheceaceae 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], *Mangifera 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 crispa*, *Lactuca esculenta*, *Lactuca scariola* L. ssp. *sativa*, *Lactuca scariola* L. var. *integrata*, *Lactuca scariola* L. var. *integrifolia*, *Lactuca sativa* subsp. *romana*, *Locusta communis*, *Valeriana locusta* [salad vegetables], *Tagetes lucida*, *Tagetes erecta* or *Tagetes tenuifolia* [african or french marigold], Apiaceae, such as the genus *Daucus*, for example the genus and species *Daucus carota* [carrot], Betulaceae, such as the genus *Corylus*, for example the genera and species *Corylus avellana* or *Corylus colurna* [hazelnut], Boraginaceae, such as the genus *Borago*, for example the genus and species *Borago officinalis* [borage], Brassicaceae, such as the genera *Brassica*, *Melanosinapis*, *Sinapis*, *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], Cryptocodiniaceae, such as the genus *Cryptocodinium*, for example the genus and species *Cryptocodinium 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 pur-*

pureus, *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 schimperii*, *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 lebbek*, *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 lebbek*, *Acacia macrophylla*, *Albizia lebbek*, *Feuillea lebbek*, *Mimosa lebbek*, *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 muhlenbergii*, *Funaria orcuttii*, *Funaria plano-convexa*, *Funaria polaris*, *Funaria ravenelii*, *Funaria rubriseta*, *Funaria serrata*, *Funaria sonorae*, *Funaria sublimbatu*, *Funaria tucsoni*, *Physcomitrella californica*, *Physcomitrella patens*, *Physcomitrella readeri*, *Physcomitrium australe*, *Physcomitrium californicum*, *Physcomitrium collenchymatum*, *Physcomitrium coloradense*,

Physcomitrium cupuliferum, *Physcomitrium drummondii*, *Physcomitrium eurystomum*, *Physcomitrium flexifolium*, *Physcomitrium hookeri*, *Physcomitrium hookeri* var. *serratum*, *Physcomitrium immersum*, *Physcomitrium kellermanii*, *Physcomitrium megalocarpum*, *Physcomitrium pyriforme*, *Physcomitrium pyriforme* var. *serratum*, *Physcomitrium rufipes*, *Physcomitrium sandbergii*, *Physcomitrium subsphaericum*, *Physcomitrium washingtoniense*, Geraniaceae, such as the genera *Pelargonium*, *Cocos*, *Oleum*, for example the genera and species *Cocos nucifera*, *Pelargonium grossularioides* or *Oleum cocois* [coconut], Gramineae, such as the genus *Saccharum*, for example the genus and species *Saccharum officinarum*, Juglandaceae, such as the genera *Juglans*, *Wallia*, for example the genera and species *Juglans regia*, *Juglans ailanthifolia*, *Juglans sieboldiana*, *Juglans cinerea*, *Wallia cinerea*, *Juglans bixbyi*, *Juglans californica*, *Juglans hindii*, *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], Lythraceae, 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 dochma*, *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*, *Rhodorus*, *Vanhoeffenia*, for example the genus and species *Porphyridium cruentum*, Proteaceae, such as the genus *Macadamia*, for example the genus and species *Macadamia integrifolia* [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. *glabriusculum*, *Capsicum frutescens* [pepper], *Capsicum annuum* [paprika], *Nicotiana tabacum*, *Nicotiana alata*, *Nicotiana attenuata*, *Nicotiana glauca*, *Nicotiana langsdorffii*, *Nicotiana obtusifolia*, *Nicotiana quadrivalvis*, *Nicotiana repanda*, *Nicotiana rustica*, *Nicotiana sylvestris* [tobacco], *Solanum tuberosum* [potato], *Solanum melongena* [eggplant], *Lycopersicon esculentum*, *Lycopersicon lycopersicum*, *Lycopersicon pyriforme*, *Solanum integrifolium* or *Solanum lycopersicum* [tomato], Sterculiaceae, such as the genus *Theobroma*, for example the genus and species *Theobroma cacao* [cacao] or Theaceae, such as the genus *Camellia*, for example the genus and species *Camellia sinensis* [tea]. In particular preferred plants to be used as transgenic plants in accordance with the present invention are oil fruit crops which comprise large amounts of lipid compounds, such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, sesame, *Calendula*, *Punica*, evening primrose, mullein, thistle, wild roses, hazelnut, almond, macadamia, avocado, bay, pumpkin/squash, linseed, soybean, pistachios, borage, trees (oil palm, coconut, walnut) or crops such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, *Tagetes*, *Solanaceae* plants such as potato, tobacco, eggplant and tomato, *Vicia* species, pea, alfalfa or bushy plants (coffee, cacao, tea), *Salix* species, and perennial grasses and fodder crops. Preferred plants according to the invention are oil crop plants such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, *Calendula*, *Punica*, evening primrose, pumpkin/squash, linseed, soybean, borage, trees (oil palm, coconut). Especially preferred are sunflower, safflower, tobacco, mullein, sesame, cotton, pumpkin/squash, poppy, evening primrose, walnut, linseed, hemp, thistle or safflower. Very especially preferred plants are plants such as safflower, sunflower, poppy, evening primrose, walnut, linseed, or hemp.

Preferred mosses are *Physcomitrella* or *Ceratodon*. Preferred algae are *Isochrysis*, *Mantoniella*, *Ostreococcus* or *Cryptocodium*, and algae/diatoms such as *Phaeodactylum*

or *Thraustochytrium*. More preferably, said algae or mosses are selected from the group consisting of: *Shewanella*, *Physcomitrella*, *Thraustochytrium*, *Fusarium*, *Phytophthora*, *Ceratodon*, *Isochrysis*, *Aleurita*, *Muscarioides*, *Mortierella*, *Phaeodactylum*, *Cryptothecodinium*, specifically from the genera and species *Thalassiosira pseudonona*, *Euglena gracilis*, *Physcomitrella patens*, *Phytophthora infestans*, *Fusarium gramineum*, *Cryptocodinium cohnii*, *Ceratodon purpureus*, *Isochrysis galbana*, *Aleurita farinosa*, *Thraustochytrium* sp., *Muscarioides viallii*, *Mortierella alpina*, *Phaeodactylum tricorutum* or *Caenorhabditis elegans* or especially advantageously *Phytophthora infestans*, *Thalassiosira pseudonona* and *Cryptocodinium cohnii*.

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

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

However, it will be understood that dependent on the non-human transgenic organism specified above, further, enzymatic activities may be conferred to the said organism, e.g., by recombinant technologies. Accordingly, the present invention, preferably, envisages a non-human transgenic organism specified above which in addition to the polynucleotide of the present invention comprises polynucleotides encoding such desaturases and/or elongases as required depending on the selected host cell. Preferred desaturases and/or elongases which shall be present in the organism are at least one enzyme selected from the group of desaturases and/or elongases or the combinations specifically recited elsewhere in this specification (see above and Tables 3, 4 and 5).

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

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

The term "polyunsaturated fatty acids (PUFA)" as used herein refers to fatty acids comprising at least two, preferably, three, four, five or six, double bonds. Moreover, it is to be understood that such fatty acids comprise, preferably from 18 to 24 carbon atoms in the fatty acid chain. More preferably, the term relates to long chain PUFA (LCPUFA) having from 20 to 24 carbon atoms in the fatty acid chain. Preferred unsaturated fatty acids in the sense of the present invention are selected from the group consisting of DGLA 20:3 (8,11,14), ARA 20:4 (5,8,11,14), iARA 20:4(8,11,14,17), EPA 20:5 (5,8,11,14,17), DPA 22:5 (4,7,10,13,16), DHA 22:6 (4,7,10,13,16,19), 20:4 (8,11,14,17), more preferably, arachidonic acid (ARA) 20:4 (5,8,11,14), eicosapentaenoic acid (EPA) 20:5 (5,8,11,14,17), and docosahexaenoic acid (DHA) 22:6 (4,7,10,13,16,19). Thus, it will be understood that most preferably, the methods provided by the present invention pertaining to the manufacture of ARA, EPA or DHA. Moreover, also encompassed are the intermediates of LCPUFA

which occur during synthesis. Such intermediates are, preferably, formed from substrates by the nECR activity of the polypeptide of the present invention. Preferably, substrates encompass LA 18:2 (9,12), GLA 18:3 (6,9,12), DGLA 20:3 (8,11,14), ARA 20:4 (5,8,11,14), eicosadienoic acid 20:2 (11,14), Eicosatetraenoic acid 20:4 (8,11,14,17), Eicosapentaenoic acid 20:5 (5,8,11,14,17).

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

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

The host cells to be used in the method of the invention are grown or cultured in the manner with which the skilled worker is familiar, depending on the host organism. Usually, host cells are grown in a liquid medium comprising a carbon source, usually in the form of sugars, a nitrogen source, usually in the form of organic nitrogen sources such as yeast extract or salts such as ammonium sulfate, trace elements such as salts of iron, manganese and magnesium and, if appropriate, vitamins, at temperatures of between 0° C. and 100° C., preferably between 10° C. and 60° C. under oxygen or anaerobic atmosphere dependent on the type of organism. The pH of the liquid medium can either be kept constant, that is to say regulated during the culturing period, or not. The cultures can be grown batchwise, semibatchwise or continuously. Nutrients can be provided at the beginning of the fermentation or administered semicontinuously or continuously: The produced PUFA or LCPUFA can be isolated from the host cells as described above by processes known to the skilled worker, e.g., by extraction, distillation, crystallization, if appropriate precipitation with salt, and/or chromatography. It might be required to disrupt the host cells prior to purification. To this end, the host cells can be disrupted beforehand. The culture medium to 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.

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

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

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

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

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

specified above. Moreover, the oil of the invention may comprise other molecular species as well. Specifically, it may comprise minor impurities of the polynucleotide or vector of the invention. Such impurities, however, can be detected only by highly sensitive techniques such as PCR.

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

FIGURES

FIG. 1 shows the nucleotide (SEQ ID NO: 1) and amino acid sequences of nECR from *Thalassiosira pseudonana* (SEQ ID NO: 2).

FIG. 2 shows the nucleotide and amino acid sequence of nECR from *Phaeodactylum tricorutum* as follows: A) the cDNA sequence of the mRNA (SEQ ID NO:3); B) the translated amino acid sequence (SEQ ID NO:4)

FIG. 3 shows an Alignment of the amino acid sequences from nECR from *T. pseudonana* and *P. tricorutum* with the dehydratase (YJL097W) and enoyl-CoA-reductase (YDL015C) from *Saccharomyces cerevisiae*

FIG. 4 shows the similarity table of the alignment from FIG. 3. The identity table was done by ClustalW alignment using the Align program from the Vector NTI software package (Invitrogen). The two nECR from the different organisms share 65% identity whereas both nECR have below 30% identity to the known dehydratase (YJL097w) or enoyl-CoA-reductase (YDL015C) from yeast.

FIG. 5 shows the functional characterization of nECR(Tp) and nECR(Pt) by yeast complementation assay. The complementation assay was performed with nECR(Tp) and nECR(Pt) in yeast *Dydl015c*, which has no functional enoyl-CoA-reductase. Legend: YPD, complete medium, SD-Ura, medium lacking uracil, SD-Leu, medium lacking leucine, FOA, medium containing leucine and 5-FOA; (1) *tsc13ΔTrp pTSC13 URA*: yeast mutant *Dydl015C* lacking the function of the enoyl-CoA-reductase, transformed with the vector *pTSC13* containing the functional *YDL015C* gene; (2) *pHs1Δkan pPHS1 URA*: yeast mutant *Δyjl097w* lacking the function of the dehydratase, transformed with the vector *pPHS1* containing the functional *YJL097W* gene; (3) *tsc13ΔTrp pESC-nECR(Tp)*: yeast mutant *Dydl015C* lacking the function of the enoyl-CoA-reductase, transformed with the vector *pESC-nECR(Tp)*; (4) *sc13ΔTrp pESC-nECR(Pt)*: yeast mutant *Dydl015C* lacking the function of the enoyl-CoA-reductase, transformed with the vector *pESC-nECR(Pt)*

FIG. 6 shows the increased production of long-chain PUFA with nECR(Tp). Increased production of long-chain PUFA with nECR(Tp). Yeast transformed with *pYES-d9Elo(Ig)* (A) or *pYES-d9Elo(Ig)+pESC-nECR(Tp)* (B) were fed with 25 μ M linolenic acid (18:3 Δ 9,12,15) in the SD(-Ura-Leu) medium. After 48 h of incubation the yeast cells were centrifuged and the pellets subjected to gas chromatographic analysis. The gas chromatographs show the different fatty acids in the two different yeast strains without (A) and with nECR(Tp) (B). Conversion rates are figured as following: (product/substrate-product)*100.

FIG. 7 shows a Western Blot analysis of *T. pseudonana* subcellular fractions. M, protein size marker, 1, total extract, 2, soluble fraction, 3, membrane fraction. The arrows indicate the two versions of nECR(Tp).

FIG. 8 shows a Western Blot analysis of subcellular fractions from yeast expressing nECR(Tp). M, protein size marker, 1, total extract, 2, soluble fraction, 3, membrane fraction. The arrow indicates the nECR(Tp) fusion protein.

FIG. 9 shows a Western Blot analysis of subcellular fractions from yeast expressing nECR(Tp). M, protein size marker, 1, total extract, 2, soluble fraction, 3, membrane fraction. The arrow indicates the nECR(Tp) fusion protein.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the figures, are incorporated herein by reference.

EXAMPLES

Example 1

Organisms and Culture Conditions

For regeneration of haploids, the method outlined in Pan et al 2004 (Molecular Cell 16:487-496) was used. Briefly, cultures were grown overnight in DOB-uracil, then approximately 25 OD₆₀₀ of each culture was washed and resuspended in fresh media and grown for 3 hours. Cells were then suspended in sporulation media (1% potassium acetate, 0.005% zinc acetate), sporulated for 5 days and spread on haploid selection magic media plates (2% galactose, amino acid mix—uracil-leucine-histidine-arginine, 0.17% nitrogen base without amino acids or ammonium sulfate, 0.1% sodium glutamate) containing 200 mg/L G418 and 60 mg/L canavanine.

Example 2

Novel Dehydratase/Enoyl-CoA-Reductase Sequences

PUFA producing algae were screened to reveal differences between non-PUFA producers. Sequences from *Thalassiosira* and *Phaeodactylum* were obtained, which are specific for these PUFA producers (FIGS. 1 and 2). Alignment with known dehydratase and enoyl-CoA-reductase sequences from yeast (YJL097W and YDL015C) showed low homology (FIGS. 3 and 4). Therefore the newly generated nECR represents a novel class of enzymes. By complementation assays (Example 3) the sequences exhibit dehydratase and enoyl-CoA-reductase activity and were therefore termed nECR.

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

TABLE 1a

List of full-length coding sequences			
SEQ ID NO:	Gene	Organism	Length in bp
1	nECR(Tp)	<i>Thalassiosira pseudonana</i>	2367
3	nECR(Pt)	<i>Phaeodactylum tricornutum</i>	2244

TABLE 1b

List of deduced amino acids from sequences described in Table 1.			
SEQ ID NO:	Gene	Organism	Length in amino acids
2	nECR(Tp)	<i>Thalassiosira pseudonana</i>	788
4	nECR(Pt)	<i>Phaeodactylum tricornutum</i>	747

Open reading frames as shown in Table 1 were cloned into the pESC(Leu) vector from Stratagene according to manufacturer's reaction conditions. Reactions were transformed into *E. coli* DH5 α and plasmid DNA was isolated. The plasmids pESC-nECR(Tp), pESC-nECR(Pt) were then used for yeast transformation. As both mutant yeast strains Δ ycl015C (Δ tsc13) and Δ yjl097w (Δ phs1) are lethal, the strains have been transformed with plasmids complementing the mutant with uracil-auxotrophic marker URA (pTSC13 and pPHS1). Vectors containing the URA marker can be removed in yeast by using 5-FOA (5-fluoroorotic acid; Sadowski et al. Yeast. 2008 Aug; 25 (8):595-9).

Example 3

Yeast Transformation and Growth Conditions

S. cerevisiae strain YSC1021-674054 from Open Biosystems was transformed with the constructs pESC-nECR(Tp), pESC-nECR(Pt) and pESC using the S. C. EasyComp Transformation Kit (Invitrogen, Carlsbad, California) with selection on leucine-deficient medium. For assessing the dehydratase/enoyl-CoA-reductase activity complementation studies were done. For that purpose the heterozygous magic marker strain YSC1021-674054 from Open Biosystems was used. This strain does not exhibit any enoyl-CoA-reductase activity. As the enoyl-CoA-reductase activity delivers elongated fatty acids and these fatty acids are required for cell growth and division, the respective yeast strain will not grow on medium not containing said elongated fatty acids.

Following Transforms were Generated:

1. tsc13 Δ Trp pTSC13 URA: yeast mutant Δ ycl015C lacking the function of the enoyl-CoA-reductase, transformed with the vector pTSC13 containing the functional YDL015C gene.
2. phs1 Δ kan pPHS1 URA: yeast mutant Δ yjl097w lacking the function of the dehydratase, transformed with the vector pPHS1 containing the functional YJL097W gene.
3. tsc13 Δ Trp pESC-nECR(Tp): yeast mutant Δ ycl015C lacking the function of the enoyl-CoA-reductase, transformed with the vector pESC-nECR(Tp)
4. sc13 Δ Trp pESC-nECR(Pt): yeast mutant Δ ycl015C lacking the function of the enoyl-CoA-reductase, transformed with the vector pESC-nECR(Pt)

Yeast were grown after transformation in complete medium containing all amino acids and nucleotides. Then yeast were plated on different medium containing either the complete medium (SD), the complete medium lacking uracil (SD-Ura), the complete medium lacking leucine (SD-Leu) or the complete medium lacking leucine and containing 5-FOA (FIG. 5). Plasmids 1 and 2 can grow on SD and SD-Ura, but not on SD-Leu as they are not having the LEU marker. Plasmids 3 and 4 can grow on SD and SD-Leu, but not on SD-Ura as they are missing the URA marker. The complementation is shown on plates containing FOA, which removes plasmids with the URA marker (1 and 2). However, even in the absence of plasmids 1 or 2 there is growth of colonies with plasmids 3 and 4 (FIG. 5, FOA).

Therefore both sequences nECR(Tp) and nECR(Pt) are capable of complementing the lethal null mutation in the yeast 3-hydroxy acyl-CoA dehydratase gene Δ ycl015C.

In summary, by complementation of a defective yeast mutant it could be shown that the sequences nECR(Tp) and nECR(Pt) are biological functional and exhibit enoyl-CoA-reductase activity.

Expression of nECR(Tp) in Combination with the d9-elongase from *Isochrysis galbana*

To examine the utility of nECR(Tp) in the production of polyunsaturated fatty acids in plants, for example, for nutraceutical use, the gene was expressed in combination with a PUFA-elongase component, the d9-elongase from *Isochrysis galbana* (WO2002/077213). This enzyme catalyzes the elongation of linoleic or linolenic acid (18:2Δ9,12 or 18:3Δ9,12, 15). The aim of the experiment was to analyze, if the addition of nECR(Tp) increases the productivity of the d9-elongase from *I. galbana*. For that purpose yeast cells (INVSC from Invitrogen) transformed with pESC-nECR(Tp) and grown on DOB(-leucine) plates were further transformed with the plasmid pYES(Ura)-d9EIo(Ig) as described in Example 1 and grown on DOB(-uracil, -leucine). The vector pYES(Ura) was derived from Invitrogen and mediates auxotrophy for uracil. The open reading frame of d9EIo(Ig) as described in WO2002/077213 was cloned into pYES2 according to manufactures conditions. As a control experiment pYES-d9EIo(Ig) was transformed into the control yeast strain containing only the pYES vector.

Surprisingly a difference in the amount of the elongation product 20:3 between the control (pESC-d9EIo(Ig) and the yeast containing two components of the elongation complex (pESC-d9EIo(Ig)+pYES-nECR(Tp) was observed.

In FIG. 6 it is shown that the addition of the nECR(Tp) gene has an tremendous influence on the productivity of long-chain PUFA. Productivity was increased 8-fold compared to the control experiment. The productivity is measured in the conversion of the substrate 18:3 (exogenously added to the yeast medium) to the elongated PUFA fatty acid 20:3.

In summary nECR improves greatly the production of elongated fatty acids, such as long-chain PUFA beneficial for human health.

Example 5

Comparison of Yeast Expressed nECR(Tp) and the Native Protein from *T. pseudonana*

Antibodies against nECR(Tp) have been produced according to manufactures practice (Eurogentec, Belgium; peptide antibody). The antibodies are highly specific for nECR(Tp) in *T. pseudonana* and the heterologous expression in yeast (FIG. 7 and FIG. 8). With the use of the antibodies structural differences between the natural organism and the heterologous expression could be observed (FIG. 7 and FIG. 8).

Western Blot experiments were Done Using Standard Protocols:

SDS-PAGE was done according to Laemmli (1970) with precast gels from Biorad. As loading buffer 0.05 M Tris/HCl pH6.8, 0.1 M DTT, 2% (w/v) SDS, 0.1% Bromphenolic blue and 10% Glycerol was used. SDS-PAGE gels were then blotted on nitrocellulose using a Protean BA85 nitrocellulose membrane (Schleicher&Schuell). Transfers on the membranes were done with a buffer containing 15 mM Na₂HPO₄ pH7.2, 0.05% (w/v) SDS, 20% (v/v) Methanol for 2 h at 200 mA, 40V (Protean II, Biorad). For the immunological test the membrane was blocked for 1 h in PBS (0.14M NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄ pH7.4), 5% (w/v) milk powder. The serum containing the antibodies against nECR(Tp) was added at a concentration of 1:2000 and incubated overnight at 4° C. For detection of the antibodies the membrane was washed three times with PBS and blocked

again with PBS, 5% (w/v) milk powder for 30 min. One unit of secondary antibody (Biorad anti-rabbit horse radish peroxidase) was added and further incubated for 30 min. After three times washing with PBS the membrane was immersed in ECL solution and 1 min incubated. Then the solution was removed with a paper tissue and the membrane was wrapped with Saran. Detection of the chemiluminescence was done in the Biolluminator (LKB).

For protein isolation from *T. pseudonana*, a culture of 500 mL was incubated for 14 days at 20° C. in F2 medium (growth conditions and media for *T. pseudonana* used as described in Tonon et al. (Tono 2005, FEBS J. 272:3401-3412). The algae was harvested by centrifugation (10 min, 5000× g) and the pellet was put into a mortar. Using a pistil a fine powder was generated. The powder was suspended with 50 mM Tris/HCl pH 8.0, 2 mM EDTA and filtered through 2 layers of Miracloth (Merck) or any other filter membrane. The filter product was then aliquoted and aliquots of 50 uL were mixed with SDS-PAGE loading buffer (see above).

Analysis of the nECR(Tp) detected by Western Blot in *T. pseudonana* (FIG. 7) showed that the protein of nECR(Tp) can be found in the cell debris, soluble and membrane fraction. Surprisingly in *T. pseudonana* the major form is a cleaved version which contains only the enoyl-CoA reductase activity (ECR). The proof for the ECR domain is deduced from the location of the binding site of the antibodies in the ECR domain. The larger fusion protein of nECR(Tp) is found only in small quantities in the soluble fraction. No nECR(Tp) could be found in the membrane fraction, even after longer exposure times.

Therefore it can be concluded that in *T. pseudonana* there are two versions of nECR(Tp), the protein as deduced from the cDNA (SEQ ID NO 1) and a post-translationally modified shorter version containing only the ECR domain. Only the ECR domain is membrane bound and therefore correctly localized (functionality of ECR in the elongase complex takes place at the microsomal membranes (Napier 2007, Annu Rev Plant Biol 58:295-319)).

With the heterologous expression of nECR(Tp) in yeast a different picture can be observed. As described in Example 4 yeast with pESC-nECR(Tp) was used for protein extraction. Yeast was grown for 3 d at 28° C. in 50 ml cultures and pellets were harvested by centrifugation (10 min, 5000× g). The pellet was aliquoted, frozen with liquid nitrogen and a steel bead added compatible with the Qiagen/Tresch mill system. Pellets were subjected to 5 min in the Tresch mill for cell disruption. Total cell extracts were separated in soluble and membrane fraction by a 30 min 100,000×g centrifugation step. The pellet constitutes the membrane fraction, the supernatant the soluble one. All three fractions were subjected to SDS-PAGE and Western analysis as described above and analyzed (FIG. 8). In yeast no cleavage of nECR(Tp) could be observed. From the molecular weight the yeast expressed fusion protein runs at approx. 86 kDa which is comparable to nECR(Tp) in *T. pseudonana*. No 59 kDa ECR cleaved version is present. Therefore it can be concluded that nECR(Tp) as it complements the yeast KO mutants (Example 3) is fully functional in it's uncleaved form, thereby representing a new class of proteins. Further, the soluble and microsomal fractions were analyzed in detail to check if there is any soluble fraction of nECR(Tp) (FIG. 9). Again supernatant and microsomal fraction was loaded on a gel and exposed for a longer time. No nECR(Tp) could be found in the soluble fraction, again showing a structural difference to the native version from *T. pseudonana*.

In conclusion a new fusion protein was discovered which surprisingly contains two enzyme activities of the elongation

complex (ECR and DH). SEQ ID NO. 1 results in the expression in heterologous systems in a functional fusion protein, which has different structural properties than the native proteins in *T. pseudonana*.

Example 6

Expression of nECR(Tp) and nECR(Pt) in Plants

The novel nECR from *T. pseudonana* and *P. tricornutum* are cloned into a plant transformation vector as described in WO2003/093482, WO20051083093 or WO2007/093776. Exemplary suitable combinations of genes are described in Table 3, 4 and 5.

TABLE 3

Gene combinations for the production of ARA.		
Gene	Aktivität	SEQ ID NO:
D6Des(Ot)	$\Delta 6$ -Desaturase	5
D6Elo(Pp)	$\Delta 6$ -Elongase	6
D5Des(Tc)	$\Delta 5$ -Desaturase	7
D12Des(Ps)	$\Delta 12$ -Desaturase	8
D6Elo(Tp)	$\Delta 6$ -Elongase	9
nECR(Tp) or nECR(Pt)	nECR	1 or 3

TABLE 4

Gene combinations for the production of EPA.		
Gene	Aktivität	SEQ ID NO:
D6Des(Ot)	$\Delta 6$ -Desaturase	5
D6Elo(Pp)	$\Delta 6$ -Elongase	6
D5Des(Tc)	$\Delta 5$ -Desaturase	7
D12Des(Ps)	$\Delta 12$ -Desaturase	8
D6Elo(Tp)	$\Delta 6$ -Elongase	9
(3-Des(Pi)	Omega 3-Desaturase	10
D15Des(Cp)	$\Delta 15$ -Desaturase	11
nECR(Tp) or nECR(Pt)	nECR	1 or 3

TABLE 5

Gene combinations for the production of DHA.		
Gene	Aktivität	SEQ ID NO:
D6Des(Ot)	$\Delta 6$ -Desaturase	5
D6Elo(Pp)	$\Delta 6$ -Elongase	6
D5Des(Tc)	$\Delta 5$ -Desaturase	7
D12Des(Ps)	$\Delta 12$ -Desaturase	8
D6Elo(Tp)	$\Delta 6$ -Elongase	9
$\omega 3$ -Des(Pi)	Omega 3-Desaturase	10
D15Des(Cp)	$\Delta 15$ -Desaturase	11
D5Elo(Ot)	$\Delta 5$ -elongase	12
D4Des(Tc)	$\Delta 4$ -desaturase	13
nECR(Tp) or nECR(Pt)	nECR	1 or 3

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

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

SEQUENCE LISTING

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<213> ORGANISM: *Thalassiosira pseudonana*

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gctatgtcta gcaagtctag cgataagttc cacactgggt ttatcaaggg tgaactcct 1140
cctcaaaaag agaagaagct cgaagttgga tacaagggaa agcagatctc tggatgag 1200
cttaaggctc aagtgaaga gtgggttgac tacggaacta tcgagccttc tgctggtgaa 1260
gctatctggt cttgcgttga taacctgga tggatcgatc tttctgacag atacttctgt 1320
cttcttgag ctggatctgc tatgggaact ttcgaggttt tgatgcaact tggagctaac 1380
gttatcggaa tcgacctcga cagacctttt atctggcaga ggcttatcaa cagagtgatg 1440
aactcttctg gatctatcac cttccctatg tctaaagaac agagcaagtg cgtgatgag 1500
aaagagcttt tcgctgcttc tggatgcaac cttttcactc aggetcctat gatcagagat 1560
tggctcgttg atctttacc tggaaagtct ttcactgtgg gatcttacgc ttacctaac 1620
ggctctctcc acgttcaagt ttctcttctg atggatgcta tctgtcgtga tctctcgat 1680
aagaggaaga acacttctct tgcttacctc tgcactccta ctgatcttca ccttatccct 1740
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atgtttatga agttgttctt cggaaagaaa accctcagaa agaactgaa gaagcctgtt 1860
agcggagttg gtggagattt ctactacgtg aacggaatct ctgttctca aggacctaac 1920
tacgctcttg ctaagagaat gcaacactgg cgtgctgtta tcgctagaag caagggatgt 1980
atcgtgtcta gcaatatcgc tcttctacc tctactgttt ctgtgactca gaacagaact 2040
ttcgttggg cttacgaggg aatgccttac ttcaagcctt acgagatctt cgtctctgag 2100
acttctaact ctgtgatgag cgctatcctt ttcaacgato tcaacaacca caagtctatc 2160
gctaaccctg atgttggaaat cgctaaccce aaccagcttt tctctttcgg tgctttccac 2220
ggtggaactt ggagatgtgc ttacagagatt gattctatcg gagaggcttc tgttcttctc 2280
tacttctctc gtgttgctaa accttacgct atcgttctcg gaggacttgg tctagctgct 2340

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ggtgctaagt ggttcggaat tgtgtga

2367

<210> SEQ ID NO 2

<211> LENGTH: 788

<212> TYPE: PRT

<213> ORGANISM: *Thalassiosira pseudonana*

<400> SEQUENCE: 2

Met Ala Ala Ala Lys Gln Gln Gln Ser Lys Gly Leu Gly Leu Lys Asp
 1 5 10 15

Leu Tyr Leu Ile Thr Tyr Asn Ala Leu Cys Cys Leu Gly Trp Ala Tyr
 20 25 30

Val Leu Ala Leu Gly Ile Pro Thr Phe Ile Ala Ser Val Thr Ser Ser
 35 40 45

Ile Gly Thr Ser Ser Leu Val Glu Ser Leu Lys Ile Ala Gly Arg Ser
 50 55 60

Val Tyr Ala Ala Thr Pro Tyr Thr Ala Gly Trp Ser Asn Glu Ala Thr
 65 70 75 80

Pro Ser Leu Ala Thr Val Leu Met Tyr Val Gln Ser Ala Ala Val Leu
 85 90 95

Glu Ile Val His Ala Ala Leu Gly Leu Val Arg Ser Pro Val Phe Val
 100 105 110

Thr Thr Met Gln Val Gly Ser Arg Ile Val Ala Leu His Met Leu Ser
 115 120 125

Thr Cys Pro Ser Ala Gln Thr Gln Trp Gly Ala Ala Leu Met Ile Phe
 130 135 140

Ser Trp Ala Leu Val Glu Val Pro Arg Tyr Leu Phe Tyr Val Ala Ala
 145 150 155 160

Ile Val Thr Gly Asp Ala Thr Lys Gly Thr Pro Tyr Pro Leu Phe Trp
 165 170 175

Leu Arg Tyr Ser Leu Phe Ala Val Leu Tyr Pro Thr Gly Ile Ser Gly
 180 185 190

Glu Leu Ser Val Phe Leu Thr Ser Ala Lys Cys Asp Thr Phe Leu Ser
 195 200 205

Thr Leu Gly Glu Ser Asn Lys Ser Ile Met Tyr Trp Tyr Ala Met Ala
 210 215 220

Phe Pro Ile Ile Tyr Ala Pro Gly Ala Leu Pro Met Ile Phe Asn Met
 225 230 235 240

Val Ala Asn Arg Lys Ser Ala Met Lys Lys Arg Phe Ala Arg Pro Pro
 245 250 255

Pro Pro Pro Arg Gly Leu Val Trp Pro Val Thr Glu Thr Lys Ala Asn
 260 265 270

Gly Glu Glu Val Arg Ser Ser Thr Pro Val Ala Lys Glu Ile Leu Ala
 275 280 285

Ala Ala Ile Gly Ala Val Asn Pro Glu Leu Ala Glu Lys Val Arg Asn
 290 295 300

Glu Lys Lys Trp Arg Phe Gly Tyr Gln Lys His Leu Val Asn Met Val
 305 310 315 320

Glu Ala Gln Cys Lys Ser Pro Glu Asp Ala Leu Lys Ile Ala Asn Ala
 325 330 335

Gly Leu Asn Lys Ala Tyr Met Thr Phe Gln Phe Val Ser Ser Asp Gly
 340 345 350

Ser Lys Thr Thr Thr Phe Ala Glu Ala Met Ser Ser Lys Ser Ser Asp
 355 360 365

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Lys Phe His Thr Gly Phe Ile Lys Gly Glu Leu Ala Pro Gln Lys Glu
 370 375 380
 Lys Lys Leu Glu Val Gly Tyr Lys Gly Lys Gln Ile Ser Gly Asp Glu
 385 390 395 400
 Leu Lys Ala Gln Val Lys Glu Trp Val Asp Tyr Gly Thr Ile Glu Pro
 405 410 415
 Ser Ala Gly Glu Ala Ile Cys Ser Cys Val Asp Asn Pro Gly Trp Ile
 420 425 430
 Asp Leu Ser Asp Arg Tyr Phe Val Leu Leu Gly Ala Gly Ser Ala Met
 435 440 445
 Gly Pro Phe Glu Val Leu Met Gln Leu Gly Ala Asn Val Ile Gly Ile
 450 455 460
 Asp Leu Ser Asp Arg Pro Phe Ile Trp Gln Arg Leu Ile Asn Arg Val Met
 465 470 475 480
 Asn Ser Ser Gly Ser Ile Thr Phe Pro Met Ser Lys Glu Gln Ser Lys
 485 490 495
 Cys Ala Asp Glu Lys Glu Leu Phe Ala Ala Ser Gly Cys Asn Leu Phe
 500 505 510
 Thr Gln Ala Pro Met Ile Arg Asp Trp Leu Val Asp Leu Tyr Pro Gly
 515 520 525
 Lys Ser Phe Thr Val Gly Ser Tyr Ala Tyr Leu Asn Gly Ala Leu His
 530 535 540
 Val Gln Val Ser Leu Ala Met Asp Ala Ile Cys Arg Asp Leu Cys Asp
 545 550 555 560
 Lys Arg Lys Asn Thr Ser Leu Ala Tyr Leu Cys Thr Pro Thr Asp Leu
 565 570 575
 His Leu Ile Pro Lys Glu Ala His Asp Ala Ala Glu Ala Asn Tyr Lys
 580 585 590
 Glu Phe Ser Lys Lys Pro Phe Cys Met Phe Met Lys Leu Phe Phe Gly
 595 600 605
 Lys Lys Thr Leu Arg Lys Asn Val Lys Lys Pro Val Ser Gly Val Gly
 610 615 620
 Gly Asp Phe Tyr Tyr Val Asn Gly Ile Ser Val Ala Gln Gly Pro Asn
 625 630 635 640
 Tyr Ala Leu Ala Lys Arg Met Gln His Trp Arg Ala Val Ile Ala Arg
 645 650 655
 Ser Lys Gly Cys Ile Val Ser Ser Asn Ile Ala Pro Ser Thr Ser Thr
 660 665 670
 Val Ser Val Thr Gln Asn Arg Thr Phe Ala Trp Ala Tyr Glu Gly Met
 675 680 685
 Pro Tyr Phe Lys Pro Tyr Glu Ile Phe Ala Pro Glu Thr Ser Asn Ser
 690 695 700
 Val Met Ser Ala Ile Leu Phe Asn Asp Leu Asn Asn His Lys Ser Ile
 705 710 715 720
 Ala Asn Pro Asp Val Gly Ile Ala Asn Pro Asn Gln Leu Phe Ser Phe
 725 730 735
 Gly Ala Phe His Gly Gly Thr Trp Arg Cys Ala Tyr Glu Ile Asp Ser
 740 745 750
 Ile Gly Glu Ala Ser Val Leu Leu Tyr Phe Ser Arg Val Ala Lys Pro
 755 760 765
 Tyr Ala Ile Ala Phe Gly Gly Leu Gly Leu Ala Ala Gly Ala Lys Trp
 770 775 780

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Phe Gly Ile Val
785

<210> SEQ ID NO 3
<211> LENGTH: 2244
<212> TYPE: DNA
<213> ORGANISM: *Phaeodactylum tricornutum*

<400> SEQUENCE: 3

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atgggactta aggacgctta cctcgtgctt tacaactctg cttgttgtgc tggctgggct    60
tatgtttggt acgctgcttg tactactatc ctcgataagg ttgcaaacca gtcacctttc    120
ggatgatgct ctgctcaagt ttacgctcac gatgatactg ctactatgct tacctacgct    180
caatctgctg ctctccttga gatccttcac gctgctcttg gacttgtag atctcctggt    240
atggtgaccg ctatgcaagt tatgtctagg atcgttgctc ttgttgcctc cgtgttctct    300
tcacaagctc aaactcaatg gggagctgga cttatgatta tctcttgggc ttcagttgaa    360
gttcctcgtt acgctttcta cgttactgct ctcttactg gtgatgctac taagaaaacc    420
cctttccctc tttctggct tcgttactct cttttcgcta tcctttacc tactggaatc    480
tgtggagagc ttactgtttt cctcgtgctt tetaaggatc aagctttcgt ggataagttc    540
ggacctcttt ctgttactct ctactctatc gttctcccta tcgtgtactt cttcggatct    600
ccttttatga ttatgaatat ggtggctaac agaaagtctg ctttcaagaa gagattcgc    660
aagcctccac ctctgcttag aggactttgt tggcctgttg atgctaaggg acagagatct    720
tctaccaacg tgaacaagac gattattgct gctgctgttg gagctgtaa cgagcaaaag    780
gctgaggcta tcagatcttg taaggcttg agattccagt acgttaagca ccttagagct    840
atggttgagg aacaatgtca aactcctgag tctgctcta agatcctca agctggactt    900
gattctgctt acgatatctt cgagttcgtt gctcctgatg gatctgctac tactttcagg    960
gaagctatgg ctgctaagaa cactgagcag ttcttcactc acgttatcaa gggagaggg    1020
aacaagctta ccaagagctg tgagatccct tacaaggggt gaatccttaa gggatgatg    1080
cttaagaagc aggttcagtc ttgggctgat tacggaacta tcgagccttc tgcctgtgct    1140
gctatcgta agtgtatcga gcacctgag tggcttgata tcagcaacag atacttcggt    1200
cttcttgagg ctggatctgc tatgggacct cttcttggtc ttatggtctc cggagetaac    1260
gttatcgctg tggatcttga tagacctaat atctggaagc gtcttatcga tctcctaga    1320
caatcttctg gaacgatcac cttccctatg aagatggacc ctgcaagtg taagaacgac    1380
gaggaaatgt tcgctcagge tggatgtaac cttttcaccg acaccctat gatcagagat    1440
tggttgatga acgtttacc tggaaagtct cttactgttg gatgctacgc ttacctgat    1500
ggtgctctcc acgttcaagt ttctcttgc atggatgcta tctgctgta tctttctgag    1560
aagaggaaga acacttctct tgcctacctc tgcactccta ctgatctca cttgatccct    1620
aaagaggctc acgatgctat gaaggctaac tacaagtctt actccgaaa gctttactgt    1680
atgatgatga accttctcag cggaggaaaag ttccttagac agaactctaa gaagcctgtt    1740
tctggaaagg gtggagagta ctacctgtg aacggaatct ctgttgcctc aggacctaac    1800
tacgctcttg ctaagagaat gcaacactgg cgtgctatca ctgctagaaa caagggatgt    1860
atcgtgtcta gcaatattgc tccttctacc tctaccgttt ctgttgttca caacagaact    1920
ttcgttggg cttacgaggg aatgccttac ttcgacctt tcgagatctt cgtcctgag    1980
acttctaacy ctgttatgct tgctctctc ttctacgatc tcaacgattc tggatcttgg    2040

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gctactccta acacttctct cggaaacctt aaccagcttt tctctcaccg atctttccac 2100
ggtggagttt ggagatgtgc ttacgaggtt gactctatcg gagaatcttc tgtgtctctc 2160
tacttcggaa gaggggctaa accttatatg gttgctgctg gtgccgtggc cgcagctggt 2220
gcagcctacg tttacgctgt gtga 2244

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<210> SEQ ID NO 4
<211> LENGTH: 747
<212> TYPE: PRT
<213> ORGANISM: Phaeodactylum tricornutum

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<400> SEQUENCE: 4

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Met Gly Leu Lys Asp Ala Tyr Leu Val Leu Tyr Asn Ser Ala Cys Cys
1 5 10 15
Ala Gly Trp Ala Tyr Val Trp Tyr Ala Ala Cys Thr Thr Ile Leu Asp
20 25 30
Lys Val Ala Asn Gln Ser Pro Phe Gly Asp Ala Ser Ala Gln Val Tyr
35 40 45
Ala His Asp Asp Thr Ala Thr Met Leu Thr Tyr Ala Gln Ser Ala Ala
50 55 60
Leu Leu Glu Ile Leu His Ala Ala Leu Gly Leu Val Arg Ser Pro Val
65 70 75 80
Met Val Thr Ala Met Gln Val Met Ser Arg Ile Val Ala Leu Val Ala
85 90 95
Leu Val Phe Ser Ser Gln Ala Gln Thr Gln Trp Gly Ala Gly Leu Met
100 105 110
Ile Ile Ser Trp Ala Ser Val Glu Val Pro Arg Tyr Ala Phe Tyr Val
115 120 125
Thr Ala Leu Leu Thr Gly Asp Ala Thr Lys Lys Thr Pro Phe Pro Leu
130 135 140
Phe Trp Leu Arg Tyr Ser Leu Phe Ala Ile Leu Tyr Pro Thr Gly Ile
145 150 155 160
Cys Gly Glu Leu Thr Val Phe Leu Ala Ala Ser Lys Asp Gln Ala Phe
165 170 175
Val Asp Lys Phe Gly Pro Leu Ser Val Thr Leu Tyr Ser Ile Val Leu
180 185 190
Pro Ile Val Tyr Phe Phe Gly Ser Pro Phe Met Ile Met Asn Met Val
195 200 205
Ala Asn Arg Lys Ser Ala Phe Lys Lys Arg Phe Ala Lys Pro Pro Pro
210 215 220
Pro Ala Arg Gly Leu Cys Trp Pro Val Asp Ala Lys Gly Gln Arg Ser
225 230 235 240
Ser Thr Asn Val Asn Lys Thr Ile Ile Ala Ala Val Gly Ala Val
245 250 255
Asn Glu Gln Lys Ala Glu Ala Ile Arg Ser Cys Lys Ala Trp Arg Phe
260 265 270
Gln Tyr Val Lys His Leu Arg Ala Met Val Glu Glu Gln Cys Gln Thr
275 280 285
Pro Glu Ser Ala Leu Lys Ile Ala Gln Ala Gly Leu Asp Ser Ala Tyr
290 295 300
Asp Ile Phe Glu Phe Val Ala Pro Asp Gly Ser Ala Thr Thr Phe Arg
305 310 315 320
Glu Ala Met Ala Ala Lys Asn Thr Glu Gln Phe Phe Thr His Val Ile
325 330 335

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Lys Gly Glu Gly Asn Lys Leu Thr Lys Glu Leu Glu Ile Pro Tyr Lys
 340 345 350

Gly Gly Ile Leu Lys Gly Asp Ala Leu Lys Lys Gln Val Gln Ser Trp
 355 360 365

Ala Asp Tyr Gly Thr Ile Glu Pro Ser Ala Gly Ala Ala Ile Val Lys
 370 375 380

Cys Ile Glu His Pro Glu Trp Leu Asp Ile Ser Asn Arg Tyr Phe Val
 385 390 395 400

Leu Leu Gly Ala Gly Ser Ala Met Gly Pro Leu Leu Val Leu Met Ala
 405 410 415

Leu Gly Ala Asn Val Ile Ala Val Asp Leu Asp Arg Pro Asn Ile Trp
 420 425 430

Lys Arg Leu Ile Asp Ile Ala Arg Gln Ser Ser Gly Thr Ile Thr Phe
 435 440 445

Pro Met Lys Met Asp Pro Ser Lys Cys Lys Asn Asp Glu Glu Met Phe
 450 455 460

Ala Gln Ala Gly Cys Asn Leu Phe Thr Asp Thr Pro Met Ile Arg Asp
 465 470 475 480

Trp Leu Met Asn Val Tyr Pro Gly Lys Ser Leu Thr Val Gly Cys Tyr
 485 490 495

Ala Tyr Leu Asp Gly Ala Leu His Val Gln Val Ser Leu Ala Met Asp
 500 505 510

Ala Ile Cys Arg Asp Leu Ser Glu Lys Arg Lys Asn Thr Ser Leu Ala
 515 520 525

Tyr Leu Cys Thr Pro Thr Asp Leu His Leu Ile Pro Lys Glu Ala His
 530 535 540

Asp Ala Met Lys Ala Asn Tyr Lys Ser Tyr Ser Gly Lys Leu Tyr Cys
 545 550 555 560

Met Met Met Asn Leu Leu Ser Gly Gly Lys Phe Leu Arg Gln Asn Ser
 565 570 575

Lys Lys Pro Val Ser Gly Lys Gly Gly Glu Tyr Tyr Leu Val Asn Gly
 580 585 590

Ile Ser Val Ala Gln Gly Pro Asn Tyr Ala Leu Ala Lys Arg Met Gln
 595 600 605

His Trp Arg Ala Ile Thr Ala Arg Asn Lys Gly Cys Ile Val Ser Ser
 610 615 620

Asn Ile Ala Pro Ser Thr Ser Thr Val Ser Val Val His Asn Arg Thr
 625 630 635 640

Phe Ala Trp Ala Tyr Glu Gly Met Pro Tyr Phe Glu Pro Phe Glu Ile
 645 650 655

Phe Ala Pro Glu Thr Ser Asn Ala Val Met Ser Ala Leu Leu Phe Tyr
 660 665 670

Asp Leu Asn Asp Ser Gly Ser Trp Ala Thr Pro Asn Thr Ser Leu Gly
 675 680 685

Asn Pro Asn Gln Leu Phe Ser His Gly Ser Phe His Gly Gly Val Trp
 690 695 700

Arg Cys Ala Tyr Glu Val Asp Ser Ile Gly Glu Ser Ser Val Leu Leu
 705 710 715 720

Tyr Phe Gly Arg Val Ala Lys Pro Tyr Met Val Ala Ala Gly Ala Val
 725 730 735

Ala Ala Ala Gly Ala Ala Tyr Val Tyr Ala Val
 740 745

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<210> SEQ ID NO 5
<211> LENGTH: 1371
<212> TYPE: DNA
<213> ORGANISM: Ostreococcus tauri

<400> SEQUENCE: 5
atgtgtgttg agaccgagaa caacgatgga atccctactg tggagatcgc ttctgatgga      60
gagagagaaa gagctgaggg taacgtgaag ttgtctgctg agaagatgga acctgctgct      120
ttggctaaga ccttcgctag aagatacgtg gttatcgagg gagttgagta cgatgtgacc      180
gatttcaaac atcctggagg aaccgtgatt ttctacgctc tctctaacac tggagctgat      240
gctactgagg ctttcaagga gttccaccac agatctagaa aggctaggaa ggctttggct      300
gctttgcctt ctgacactgc taagaccgct aaagtggatg atgctgagat gctccaggat      360
ttcgctaagt ggagaaagga gttggagagg gacggattct tcaagccttc tctgctcat      420
gttgcttaca gattcgcctg gttggctgct atgtacgctt tgggaaccta cttgatgtac      480
gctagatacg ttgtgtcctc tgtgttggtt tacgcttget tcttcggagc tagatgtgga      540
tgggttcaac atgagggagg acattcttct ttgaccggaa acatctggtg ggataagaga      600
atccaagctt tcaactgctg attcggattg gctggatctg gagatatgtg gaaactccatg      660
cacaacaagc accatgctac tctcaaaaa gtgaggcacc atatggattt ggataccact      720
cctgctgttg ctttcttcaa caccgctgtg gaggataata gacctagggg attctctaag      780
tactggctca gattgcaagc ttggaccttc attcctgtga cttctggatt ggtggtgctc      840
ttctggatgt tcttctccca tcttctaag gctttgaagg gagaaaagta cgaggagctt      900
gtgtggatgt tggctgctca tgtgattaga acctggacca ttaaggctgt tactggatc      960
accgctatgc aatcctacgg actcttcttg gctacttctt gggtttccgg atgctacttg     1020
ttcgctcact tctctacttc tcacacccat ttggatgttg ttctgctga tgagcatttg     1080
tcttgggtta ggtacgctgt ggatcacacc attgatatcg atccttctca gggatgggtt     1140
aactggttga tgggataactt gaaactgcaa gtgattcacc acctcttccc ttctatgect     1200
caattcagac aacctgaggt gtccagaaga ttcggtgctt tcgctaagaa gtggaacctc     1260
aactacaagg tgatgactta tgctggagct tgggaaggcta ctttgggaaa cctcgataat     1320
gtgggaaagc actactacgt gcaccggacaa cattctgtaa agaccgcttg a              1371

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<210> SEQ ID NO 6
<211> LENGTH: 456
<212> TYPE: PRT
<213> ORGANISM: Ostreococcus tauri

<400> SEQUENCE: 6
Met Cys Val Glu Thr Glu Asn Asn Asp Gly Ile Pro Thr Val Glu Ile
1          5          10          15
Ala Phe Asp Gly Glu Arg Glu Arg Ala Glu Ala Asn Val Lys Leu Ser
20          25          30
Ala Glu Lys Met Glu Pro Ala Ala Leu Ala Lys Thr Phe Ala Arg Arg
35          40          45
Tyr Val Val Ile Glu Gly Val Glu Tyr Asp Val Thr Asp Phe Lys His
50          55          60
Pro Gly Gly Thr Val Ile Phe Tyr Ala Leu Ser Asn Thr Gly Ala Asp
65          70          75          80
Ala Thr Glu Ala Phe Lys Glu Phe His His Arg Ser Arg Lys Ala Arg
85          90          95

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Lys Ala Leu Ala Ala Leu Pro Ser Arg Pro Ala Lys Thr Ala Lys Val
 100 105 110
 Asp Asp Ala Glu Met Leu Gln Asp Phe Ala Lys Trp Arg Lys Glu Leu
 115 120 125
 Glu Arg Asp Gly Phe Phe Lys Pro Ser Pro Ala His Val Ala Tyr Arg
 130 135 140
 Phe Ala Glu Leu Ala Ala Met Tyr Ala Leu Gly Thr Tyr Leu Met Tyr
 145 150 155 160
 Ala Arg Tyr Val Val Ser Ser Val Leu Val Tyr Ala Cys Phe Phe Gly
 165 170 175
 Ala Arg Cys Gly Trp Val Gln His Glu Gly Gly His Ser Ser Leu Thr
 180 185 190
 Gly Asn Ile Trp Trp Asp Lys Arg Ile Gln Ala Phe Thr Ala Gly Phe
 195 200 205
 Gly Leu Ala Gly Ser Gly Asp Met Trp Asn Ser Met His Asn Lys His
 210 215 220
 His Ala Thr Pro Gln Lys Val Arg His Asp Met Asp Leu Asp Thr Thr
 225 230 235 240
 Pro Ala Val Ala Phe Phe Asn Thr Ala Val Glu Asp Asn Arg Pro Arg
 245 250 255
 Gly Phe Ser Lys Tyr Trp Leu Arg Leu Gln Ala Trp Thr Phe Ile Pro
 260 265 270
 Val Thr Ser Gly Leu Val Leu Leu Phe Trp Met Phe Phe Leu His Pro
 275 280 285
 Ser Lys Ala Leu Lys Gly Gly Lys Tyr Glu Glu Leu Val Trp Met Leu
 290 295 300
 Ala Ala His Val Ile Arg Thr Trp Thr Ile Lys Ala Val Thr Gly Phe
 305 310 315 320
 Thr Ala Met Gln Ser Tyr Gly Leu Phe Leu Ala Thr Ser Trp Val Ser
 325 330 335
 Gly Cys Tyr Leu Phe Ala His Phe Ser Thr Ser His Thr His Leu Asp
 340 345 350
 Val Val Pro Ala Asp Glu His Leu Ser Trp Val Arg Tyr Ala Val Asp
 355 360 365
 His Thr Ile Asp Ile Asp Pro Ser Gln Gly Trp Val Asn Trp Leu Met
 370 375 380
 Gly Tyr Leu Asn Cys Gln Val Ile His His Leu Phe Pro Ser Met Pro
 385 390 395 400
 Gln Phe Arg Gln Pro Glu Val Ser Arg Arg Phe Val Ala Phe Ala Lys
 405 410 415
 Lys Trp Asn Leu Asn Tyr Lys Val Met Thr Tyr Ala Gly Ala Trp Lys
 420 425 430
 Ala Thr Leu Gly Asn Leu Asp Asn Val Gly Lys His Tyr Tyr Val His
 435 440 445
 Gly Gln His Ser Gly Lys Thr Ala
 450 455

<210> SEQ ID NO 7

<211> LENGTH: 873

<212> TYPE: DNA

<213> ORGANISM: Physcomitrella patens

<400> SEQUENCE: 7

atggaagtgtg ttgagaggtt ctacggagag ttggatggaa aggtttccca aggagtgaac 60

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gctttgttgg gatctttcgg agttgagttg actgataccc caactactaa gggattgcca 120
ctcgttgatt ctccaactcc aattgtgttg ggagtgctg tttacttgac catcgtgatc 180
ggaggattgc tttggatcaa ggctagagat ctcaagccaa gagcttctga gccattcttg 240
ttgcaagctt tgggtgttgg gcacaacttg ttctgcttcg ctttgtctct ttacatgtgc 300
gtgggtatcg cttaccaage tatcacctgg agatattcct tgtggggaaa cgcttataac 360
ccaaagcaca aggagatggc tatcctcgtt tacctcttct acatgtccaa gtacgtggag 420
ttcatggata cctgtgatcat gatcctcaag agatccacca gacagatttc tttcctccac 480
gtgtaccacc attcctctat ctcccttacc tgggtgggcta ttgctcatca tgctccagga 540
ggagaggctt attggagtgc tgctctcaac tctggagtgc atgtgttgat gtacgcttac 600
tacttcttgg ctgcttgctt gagatcttcc ccaaagctca agaacaagta cctcttctgg 660
ggaagatacc tcaccaactt ccagatgttc cagttcatgc tcaacttggg gcaagcttac 720
tacgatatga aaaccaacgc tccatatcca caatggetca tcaagatcct cttctactac 780
atgatctccc tcttgttctt cttcggaaac ttctacgtgc aaaagtacat caagccatcc 840
gatggaaagc aaaagggagc taagaccgag tga 873

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<210> SEQ ID NO 8
<211> LENGTH: 290
<212> TYPE: PRT
<213> ORGANISM: Physcomitrella patens

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<400> SEQUENCE: 8

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Met Glu Val Val Glu Arg Phe Tyr Gly Glu Leu Asp Gly Lys Val Ser
1          5          10          15
Gln Gly Val Asn Ala Leu Leu Gly Ser Phe Gly Val Glu Leu Thr Asp
20          25          30
Thr Pro Thr Thr Lys Gly Leu Pro Leu Val Asp Ser Pro Thr Pro Ile
35          40          45
Val Leu Gly Val Ser Val Tyr Leu Thr Ile Val Ile Gly Gly Leu Leu
50          55          60
Trp Ile Lys Ala Arg Asp Leu Lys Pro Arg Ala Ser Glu Pro Phe Leu
65          70          75          80
Leu Gln Ala Leu Val Leu Val His Asn Leu Phe Cys Phe Ala Leu Ser
85          90          95
Leu Tyr Met Cys Val Gly Ile Ala Tyr Gln Ala Ile Thr Trp Arg Tyr
100         105         110
Ser Leu Trp Gly Asn Ala Tyr Asn Pro Lys His Lys Glu Met Ala Ile
115         120         125
Leu Val Tyr Leu Phe Tyr Met Ser Lys Tyr Val Glu Phe Met Asp Thr
130         135         140
Val Ile Met Ile Leu Lys Arg Ser Thr Arg Gln Ile Ser Phe Leu His
145         150         155         160
Val Tyr His His Ser Ser Ile Ser Leu Ile Trp Trp Ala Ile Ala His
165         170         175
His Ala Pro Gly Gly Glu Ala Tyr Trp Ser Ala Ala Leu Asn Ser Gly
180         185         190
Val His Val Leu Met Tyr Ala Tyr Tyr Phe Leu Ala Ala Cys Leu Arg
195         200         205
Ser Ser Pro Lys Leu Lys Asn Lys Tyr Leu Phe Trp Gly Arg Tyr Leu
210         215         220
Thr Gln Phe Gln Met Phe Gln Phe Met Leu Asn Leu Val Gln Ala Tyr

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<210> SEQ ID NO 11
 <211> LENGTH: 1197
 <212> TYPE: DNA
 <213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 11

```

atggctattt tgaaccctga ggctgattct gctgctaacc tcgctactga ttctgaggct    60
aagcaaagac aattggctga ggctggatac actcatgttg aggggtgctcc tgctccttg    120
cctttggagt tgcctcattt ctctctcaga gatctcagag ctgctattcc taagcactgc    180
ttcgagagat ctttcgtgac ctccacctac tacatgatca agaacgtggt gacttgcgct    240
gctttgttct acgctgctac cttcattgat agagctggag ctgctgctta tgttttgggg    300
cctgtgtact ggttcttcca gggatcttac ttgactggag tgtgggttat cgctcatgag    360
tgtggacatc aggccttattg ctctctgag gtgggtgaaca acttgattgg actcgtgttg    420
cattctgctt tgttggtgcc ttaccactct tggagaatct ctacagaaa gcaccattcc    480
aacactggat cttgcgagaa cgatgagggtt ttcgttcctg tgaccagatc tgtgttggtc    540
tcttcttggg acgagacctt ggaggattct cctctctacc aactctaccg tatcgtgtac    600
atgttggttg ttggatggat gcttggatac ctcttcttca acgctactgg acctactaag    660
tactggggaa agtctaggto tcaactcaac ccttactccg ctatctatgc tgataggagg    720
agatggatga tcgtgctctc cgatattttc ttggtggcta tgttggctgt tttggctgct    780
ttggtgcaca ctttctcctt caacaccatg gtgaagtctt acgtggtgcc ttacttcatt    840
gtgaacgctt acttgggtgt gattacctac ctccaacaca cggataccta catccctcat    900
ttcagagagg gagagtggaa ttggttgaga ggagctttgt gcactgtgga tagatcattt    960
ggtcatttcc tcgattctgt ggtgcataga atcgtggata cccatgtttg ccaccacatc   1020
ttctccaaga tgcctttcta tcattgagag gaggctacca acgctattaa gcctctcctc   1080
ggaaagtctt acttgaagga taccactcct gttcctgttg ctctctggag atcttacacc   1140
cattgcaagt tcgttgagga tgatggaaag gtggtgttct acaagaacaa gctctag    1197

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<210> SEQ ID NO 12
 <211> LENGTH: 398
 <212> TYPE: PRT
 <213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 12

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Met Ala Ile Leu Asn Pro Glu Ala Asp Ser Ala Ala Asn Leu Ala Thr
1           5           10           15
Asp Ser Glu Ala Lys Gln Arg Gln Leu Ala Glu Ala Gly Tyr Thr His
20          25          30
Val Glu Gly Ala Pro Ala Pro Leu Pro Leu Glu Leu Pro His Phe Ser
35          40          45
Leu Arg Asp Leu Arg Ala Ala Ile Pro Lys His Cys Phe Glu Arg Ser
50          55          60
Phe Val Thr Ser Thr Tyr Tyr Met Ile Lys Asn Val Leu Thr Cys Ala
65          70          75          80
Ala Leu Phe Tyr Ala Ala Thr Phe Ile Asp Arg Ala Gly Ala Ala Ala
85          90          95
Tyr Val Leu Trp Pro Val Tyr Trp Phe Phe Gln Gly Ser Tyr Leu Thr
100         105         110
Gly Val Trp Val Ile Ala His Glu Cys Gly His Gln Ala Tyr Cys Ser
115        120        125

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Ser Glu Val Val Asn Asn Leu Ile Gly Leu Val Leu His Ser Ala Leu
 130 135 140

Leu Val Pro Tyr His Ser Trp Arg Ile Ser His Arg Lys His His Ser
 145 150 155 160

Asn Thr Gly Ser Cys Glu Asn Asp Glu Val Phe Val Pro Val Thr Arg
 165 170 175

Ser Val Leu Ala Ser Ser Trp Asn Glu Thr Leu Glu Asp Ser Pro Leu
 180 185 190

Tyr Gln Leu Tyr Arg Ile Val Tyr Met Leu Val Val Gly Trp Met Pro
 195 200 205

Gly Tyr Leu Phe Phe Asn Ala Thr Gly Pro Thr Lys Tyr Trp Gly Lys
 210 215 220

Ser Arg Ser His Phe Asn Pro Tyr Ser Ala Ile Tyr Ala Asp Arg Glu
 225 230 235 240

Arg Trp Met Ile Val Leu Ser Asp Ile Phe Leu Val Ala Met Leu Ala
 245 250 255

Val Leu Ala Ala Leu Val His Thr Phe Ser Phe Asn Thr Met Val Lys
 260 265 270

Phe Tyr Val Val Pro Tyr Phe Ile Val Asn Ala Tyr Leu Val Leu Ile
 275 280 285

Thr Tyr Leu Gln His Thr Asp Thr Tyr Ile Pro His Phe Arg Glu Gly
 290 295 300

Glu Trp Asn Trp Leu Arg Gly Ala Leu Cys Thr Val Asp Arg Ser Phe
 305 310 315 320

Gly Pro Phe Leu Asp Ser Val Val His Arg Ile Val Asp Thr His Val
 325 330 335

Cys His His Ile Phe Ser Lys Met Pro Phe Tyr His Cys Glu Glu Ala
 340 345 350

Thr Asn Ala Ile Lys Pro Leu Leu Gly Lys Phe Tyr Leu Lys Asp Thr
 355 360 365

Thr Pro Val Pro Val Ala Leu Trp Arg Ser Tyr Thr His Cys Lys Phe
 370 375 380

Val Glu Asp Asp Gly Lys Val Val Phe Tyr Lys Asn Lys Leu
 385 390 395

<210> SEQ ID NO 13
 <211> LENGTH: 819
 <212> TYPE: DNA
 <213> ORGANISM: Thalassiosira pseudonana

<400> SEQUENCE: 13

atggatgctt ataacgctgc tatggataag attggagctg ctatcatcga ttggagtgat 60

ccagatggaa agttcagagc tgatagggag gattggtggt tgtgcgattt cagatccgct 120

atcaccattg ctctcatcta catcgctttc gtgatcttgg gatctgctgt gatgcaatct 180

ctcccageta tggacceata ccctatcaag ttctctaca acgtgtctca aatcttctc 240

tgcgcttaca tgactgttga ggctggatc ctcgcttata ggaacggata caccgttatg 300

ccatgcaacc acttcaacgt gaacgatcca ccagttgcta acttgctctg gctcttctac 360

atctccaaag tgtgggattt ctggataacc atcttcattg tgctcggaaa gaagtggaga 420

caactctctt tcttgcaagt gtaccatcat accaccatct tctcttctta ctggttgaac 480

gctaactgct tctacgatgg agatatcttc ttgaccatcc tctcacaagg attcattcac 540

accgtgatgt acacctacta cttcatctgc atgcacacca aggattctaa gaccggaaa 600

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tctttgccaa tctggtgaa gtcactttg accgctttcc aactcttgca attcaccatc 660
atgatgtccc aagctacctt cttgggtttc cacggatgcg ataaggtttc cctcagaatc 720
accatcgtgt acttcgtgta cattctctcc cttttcttcc tcttcgetca gttcttcgtg 780
caatcctaca tggctccaaa gaagaagaag tccgcttga 819

<210> SEQ ID NO 14
<211> LENGTH: 272
<212> TYPE: PRT
<213> ORGANISM: *Thalassiosira pseudonana*

<400> SEQUENCE: 14

Met Asp Ala Tyr Asn Ala Ala Met Asp Lys Ile Gly Ala Ala Ile Ile
1 5 10 15
Asp Trp Ser Asp Pro Asp Gly Lys Phe Arg Ala Asp Arg Glu Asp Trp
20 25 30
Trp Leu Cys Asp Phe Arg Ser Ala Ile Thr Ile Ala Leu Ile Tyr Ile
35 40 45
Ala Phe Val Ile Leu Gly Ser Ala Val Met Gln Ser Leu Pro Ala Met
50 55 60
Asp Pro Tyr Pro Ile Lys Phe Leu Tyr Asn Val Ser Gln Ile Phe Leu
65 70 75 80
Cys Ala Tyr Met Thr Val Glu Ala Gly Phe Leu Ala Tyr Arg Asn Gly
85 90 95
Tyr Thr Val Met Pro Cys Asn His Phe Asn Val Asn Asp Pro Pro Val
100 105 110
Ala Asn Leu Leu Trp Leu Phe Tyr Ile Ser Lys Val Trp Asp Phe Trp
115 120 125
Asp Thr Ile Phe Ile Val Leu Gly Lys Lys Trp Arg Gln Leu Ser Phe
130 135 140
Leu His Val Tyr His His Thr Thr Ile Phe Leu Phe Tyr Trp Leu Asn
145 150 155 160
Ala Asn Val Leu Tyr Asp Gly Asp Ile Phe Leu Thr Ile Leu Leu Asn
165 170 175
Gly Phe Ile His Thr Val Met Tyr Thr Tyr Tyr Phe Ile Cys Met His
180 185 190
Thr Lys Asp Ser Lys Thr Gly Lys Ser Leu Pro Ile Trp Trp Lys Ser
195 200 205
Ser Leu Thr Ala Phe Gln Leu Leu Gln Phe Thr Ile Met Met Ser Gln
210 215 220
Ala Thr Tyr Leu Val Phe His Gly Cys Asp Lys Val Ser Leu Arg Ile
225 230 235 240
Thr Ile Val Tyr Phe Val Tyr Ile Leu Ser Leu Phe Phe Leu Phe Ala
245 250 255
Gln Phe Phe Val Gln Ser Tyr Met Ala Pro Lys Lys Lys Lys Ser Ala
260 265 270

<210> SEQ ID NO 15
<211> LENGTH: 1086
<212> TYPE: DNA
<213> ORGANISM: *Phytophthora infestans*

<400> SEQUENCE: 15

atggcgacga aggaggcgta tgtgttcccc actctgacgg agatcaagcg gtcgctacct 60
aaagactgtt tcgaggcttc ggtgctctcg tcgctctact acaccgtgcg ttgtctggtg 120

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atcgcggtgg ctctaacctt cggcttcaac tacgctcgcg ctctgcccga ggtcgagagc 180
ttctgggctc tggacgcccgc actctgcaac ggctacatct tgetgcaggg catcgtgttc 240
tggggcttct tcacgggtggg ccacgatgcc ggccacggcg ccttctcgcg ctaccacctg 300
cttaacttcg tggtgggcac ttctatgcac tgcctcatcc tcacgcccctt cgagtcgtgg 360
aagctcacgc accgtcacca ccacaagaac acgggcaaca ttgacctga cgaggtcttc 420
taccgcgaac gcaaggccga cgaccaccgc ctgtctcgca acctgattct ggcgctcggg 480
gcagcgtggc tegcctatct ggctcgagggc ttccctcctc gtaaggtaaa ccacttcaac 540
cogttcgagc ctctgttctg gcgtcaggtg tcagctgtgg taatctctct tctcgcccac 600
ttctctgtgg ccggactctc catctatctg agcctccagc tgggccttaa gacgatggca 660
atctactact atggacctgt ttttgtgttc ggcagcatgc tggtcattac caccttctca 720
caccacaatg atgaggagac cccatggtac gccgactcgg agtggacgta cgtcaagggc 780
aacctctcgt ccgtggaccg atcgtacggc gcgctcattg acaacctgag ccacaacatc 840
ggcacgcacc agatccacca ccttttccct atcattccgc actacaaact caagaagcc 900
actgcgccct tccaccaggc tttccctgag ctctgtcgcga agagcgacga gcccaattatc 960
aaggttttct tccgggttgg acgtctctac gcaaactacg gcgttgtgga ccaggaggcg 1020
aagctcttca cgctaaagga agccaagggc gcgaccgagg cggcgggccaa gaccaagtcc 1080
acgtaa 1086

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<210> SEQ ID NO 16

<211> LENGTH: 361

<212> TYPE: PRT

<213> ORGANISM: Phytophthora infestans

<400> SEQUENCE: 16

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Met Ala Thr Lys Glu Ala Tyr Val Phe Pro Thr Leu Thr Glu Ile Lys
1           5           10           15
Arg Ser Leu Pro Lys Asp Cys Phe Glu Ala Ser Val Pro Leu Ser Leu
20          25          30
Tyr Tyr Thr Val Arg Cys Leu Val Ile Ala Val Ala Leu Thr Phe Gly
35          40          45
Leu Asn Tyr Ala Arg Ala Leu Pro Glu Val Glu Ser Phe Trp Ala Leu
50          55          60
Asp Ala Ala Leu Cys Thr Gly Tyr Ile Leu Leu Gln Gly Ile Val Phe
65          70          75          80
Trp Gly Phe Phe Thr Val Gly His Asp Ala Gly His Gly Ala Phe Ser
85          90          95
Arg Tyr His Leu Leu Asn Phe Val Val Gly Thr Phe Met His Ser Leu
100         105         110
Ile Leu Thr Pro Phe Glu Ser Trp Lys Leu Thr His Arg His His His
115        120        125
Lys Asn Thr Gly Asn Ile Asp Arg Asp Glu Val Phe Tyr Pro Gln Arg
130        135        140
Lys Ala Asp Asp His Pro Leu Ser Arg Asn Leu Ile Leu Ala Leu Gly
145        150        155        160
Ala Ala Trp Leu Ala Tyr Leu Val Glu Gly Phe Pro Pro Arg Lys Val
165        170        175
Asn His Phe Asn Pro Phe Glu Pro Leu Phe Val Arg Gln Val Ser Ala
180        185        190

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Val Val Ile Ser Leu Leu Ala His Phe Phe Val Ala Gly Leu Ser Ile
 195 200 205

Tyr Leu Ser Leu Gln Leu Gly Leu Lys Thr Met Ala Ile Tyr Tyr Tyr
 210 215 220

Gly Pro Val Phe Val Phe Gly Ser Met Leu Val Ile Thr Thr Phe Leu
 225 230 235 240

His His Asn Asp Glu Glu Thr Pro Trp Tyr Ala Asp Ser Glu Trp Thr
 245 250 255

Tyr Val Lys Gly Asn Leu Ser Ser Val Asp Arg Ser Tyr Gly Ala Leu
 260 265 270

Ile Asp Asn Leu Ser His Asn Ile Gly Thr His Gln Ile His His Leu
 275 280 285

Phe Pro Ile Ile Pro His Tyr Lys Leu Lys Lys Ala Thr Ala Ala Phe
 290 295 300

His Gln Ala Phe Pro Glu Leu Val Arg Lys Ser Asp Glu Pro Ile Ile
 305 310 315 320

Lys Ala Phe Phe Arg Val Gly Arg Leu Tyr Ala Asn Tyr Gly Val Val
 325 330 335

Asp Gln Glu Ala Lys Leu Phe Thr Leu Lys Glu Ala Lys Ala Ala Thr
 340 345 350

Glu Ala Ala Ala Lys Thr Lys Ser Thr
 355 360

<210> SEQ ID NO 17
 <211> LENGTH: 1434
 <212> TYPE: DNA
 <213> ORGANISM: Claviceps purpurea

<400> SEQUENCE: 17

atggctgcta ctacctctgc tatgagcaag gatgctgttc ttagaagaac tgctgctgct 60
 actactgcta tcgatcacga aagctctacc tctgcttctc cagctgattc tcttagactc 120
 tctgcttctt ctacctctct ctcttctctc agctctctcg acgctaagga taaggatgat 180
 gagtacgctg gacttcttga tacttacgga aacgctttca cccctcctga tttcactatc 240
 aaggatatca gagatgctat ccctaagcac tgcttcgagc gttctgctat caagggatac 300
 gcttatatcc tcagagatgt ggcttgctt tetaccactt totacctctt ccacaacttc 360
 gttaccctcg agaacgttcc ttacaccctt cttagagttt tctctgagg agtttacct 420
 gctcttcagg gacttttcgg aactggactc tggattatcg ctcacgagtg tggacacggt 480
 gctttctctc cttctacct cactaacgat cttactggat gggttctcca ctctgctctt 540
 ctcgtgcctt acttctcttg gaagttctct cactctgctc accacaaggg aaccggaaat 600
 atggaaaagg atatggcttt cctccctaga actagggctc aatacgtac cagattcggga 660
 agagctatgg atcagcttgg agatctttgc gaggaaccc ctatctacac tgctggatcc 720
 cttgttttcc agcagcttct tggatggcct tcttacttga tcgctaactg tactggacac 780
 gatcttcacg agagacagag agaggaaga ggaaggaa agaagaacgg attcggagga 840
 actgttaacc acttcgacc tcgttctctc atcttcgatg acaagcacgc taagtttatc 900
 gttctcagcg atatcggaact tggacttgct atcgctgctc ttgtttacct cggaaacaga 960
 ttcggatggg ctaacgttgc tgtttggtac ttcgttctt acccttggtg taaccactgg 1020
 atcgttgcta tcactttctc tcagcacact gatcctactc ttcctcacta cactgctgag 1080
 gaatggaact tcgttcgtgg agctgctgct acaatcgata gagagatggg atttatcggt 1140

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agacacctct tccacggaat cgttgagact cacgtgcttc accactacgt ttcttcaatc 1200
cctttctaca acgctgatga ggcttctgag gctatcaagc ctggttatggg aaagcactac 1260
cgttctgaga ctaaggatgg acctatgggt tttatcaggg ctttgggaa aactgctaga 1320
tgggtgcaat gggttgagcc ttctgctgat gctcaagggt ctggtgaagg tgttctcttc 1380
ttcaggaaca gaaacggact tggaactaag cctatctcta tgaggacca gtga 1434

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<210> SEQ ID NO 18

<211> LENGTH: 477

<212> TYPE: PRT

<213> ORGANISM: *Claviceps purpurea*

<400> SEQUENCE: 18

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Met Ala Ala Thr Thr Ser Ala Met Ser Lys Asp Ala Val Leu Arg Arg
1           5           10           15
Thr Ala Ala Ala Thr Thr Ala Ile Asp His Glu Ser Ser Thr Ser Ala
20           25           30
Ser Pro Ala Asp Ser Pro Arg Leu Ser Ala Ser Ser Thr Ser Leu Ser
35           40           45
Ser Leu Ser Ser Leu Asp Ala Lys Asp Lys Asp Asp Glu Tyr Ala Gly
50           55           60
Leu Leu Asp Thr Tyr Gly Asn Ala Phe Thr Pro Pro Asp Phe Thr Ile
65           70           75           80
Lys Asp Ile Arg Asp Ala Ile Pro Lys His Cys Phe Glu Arg Ser Ala
85           90           95
Ile Lys Gly Tyr Ala Tyr Ile Leu Arg Asp Val Ala Cys Leu Ser Thr
100          105          110
Thr Phe Tyr Leu Phe His Asn Phe Val Thr Pro Glu Asn Val Pro Tyr
115          120          125
Thr Pro Leu Arg Val Phe Leu Trp Gly Val Tyr Thr Ala Leu Gln Gly
130          135          140
Leu Phe Gly Thr Gly Leu Trp Ile Ile Ala His Glu Cys Gly His Gly
145          150          155          160
Ala Phe Ser Pro Ser Thr Leu Thr Asn Asp Leu Thr Gly Trp Val Leu
165          170          175
His Ser Ala Leu Leu Val Pro Tyr Phe Ser Trp Lys Phe Ser His Ser
180          185          190
Ala His His Lys Gly Thr Gly Asn Met Glu Arg Asp Met Ala Phe Leu
195          200          205
Pro Arg Thr Arg Ala Gln Tyr Ala Thr Arg Phe Gly Arg Ala Met Asp
210          215          220
Gln Leu Gly Asp Leu Cys Glu Glu Thr Pro Ile Tyr Thr Ala Gly Phe
225          230          235          240
Leu Val Phe Gln Gln Leu Leu Gly Trp Pro Ser Tyr Leu Ile Ala Asn
245          250          255
Val Thr Gly His Asp Leu His Glu Arg Gln Arg Glu Gly Arg Gly Lys
260          265          270
Gly Lys Lys Asn Gly Phe Gly Gly Thr Val Asn His Phe Asp Pro Arg
275          280          285
Ser Pro Ile Phe Asp Asp Lys His Ala Lys Phe Ile Val Leu Ser Asp
290          295          300
Ile Gly Leu Gly Leu Ala Ile Ala Ala Leu Val Tyr Leu Gly Asn Arg
305          310          315          320
Phe Gly Trp Ala Asn Val Ala Val Trp Tyr Phe Val Pro Tyr Leu Trp

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	325		330		335										
Val	Asn	His	Trp	Ile	Val	Ala	Ile	Thr	Phe	Leu	Gln	His	Thr	Asp	Pro
	340							345					350		
Thr	Leu	Pro	His	Tyr	Thr	Ala	Glu	Glu	Trp	Asn	Phe	Val	Arg	Gly	Ala
	355						360					365			
Ala	Ala	Thr	Ile	Asp	Arg	Glu	Met	Gly	Phe	Ile	Gly	Arg	His	Leu	Phe
	370					375					380				
His	Gly	Ile	Val	Glu	Thr	His	Val	Leu	His	His	Tyr	Val	Ser	Ser	Ile
	385				390					395					400
Pro	Phe	Tyr	Asn	Ala	Asp	Glu	Ala	Ser	Glu	Ala	Ile	Lys	Pro	Val	Met
			405						410					415	
Gly	Lys	His	Tyr	Arg	Ser	Glu	Thr	Lys	Asp	Gly	Pro	Met	Gly	Phe	Ile
			420					425					430		
Arg	Ala	Leu	Trp	Lys	Thr	Ala	Arg	Trp	Cys	Gln	Trp	Val	Glu	Pro	Ser
	435					440						445			
Ala	Asp	Ala	Gln	Gly	Ala	Gly	Glu	Gly	Val	Leu	Phe	Phe	Arg	Asn	Arg
	450				455						460				
Asn	Gly	Leu	Gly	Thr	Lys	Pro	Ile	Ser	Met	Arg	Thr	Gln			
	465				470					475					

<210> SEQ ID NO 19
 <211> LENGTH: 903
 <212> TYPE: DNA
 <213> ORGANISM: *Ostreococcus tauri*

<400> SEQUENCE: 19

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atgtctgctt ctggagcttt gttgctctgt attgctttcg ctgcttacgc ttacgctacc    60
tacgcttatg ctttcgagtg gtctcatgct aacggaatcg ataacgtgga tgctagagag    120
tggattggag ctttgtcttt gagactccct gcaattgcta ccaccatgta cctcttgctc    180
tgccttgtag gacctagatt gatggctaag agggaggctt ttgatcctaa gggattcatg    240
ctcgcctaca acgcctacca aaccgctttc aacgttgtag tgctcggaat gttcgctaga    300
gagatctctg gattgggaca acctgcttgg ggatctacta tgccttggag cgataggaag    360
tccttcaaga ttttgctggg agtggtgctc cattacaaca ataagtaact cgagttgctg    420
gatactgtgt tcactgtggc taggaaaaag accaagcagc tctctttctt gcatgtgtac    480
catcatgctt tgttgatttg ggcttggtag cttgtttgtc atctcatggc taccaacgat    540
tgcacatgat cttatttcgg agctgcttgc aactctttca tccacatcgt gatgtactcc    600
tactacctca tgtctgcttt gggaaattaga tgcccttggg agagatatat caccaggct    660
cagatgttgc aattcgtgat cgtgttcgct catgctgttt tcgtgctcag acaaaagcac    720
tgccctgtta ctttgccctg ggcacaaatg ttctgtgatga caaatatggt ggtgctcttc    780
ggaaacttct acctcaaggc ttactctaac aagtctaggg gagatggagc ttcttctggt    840
aagcctgctg agactactag agcaccttct gtgagaagaa ccagggtccag gaagatcgat    900
tga
    903
    
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<210> SEQ ID NO 20
 <211> LENGTH: 300
 <212> TYPE: PRT
 <213> ORGANISM: *Ostreococcus tauri*

<400> SEQUENCE: 20

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Met Ser Ala Ser Gly Ala Leu Leu Pro Ala Ile Ala Phe Ala Ala Tyr
1           5           10           15
    
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Ala Tyr Ala Thr Tyr Ala Tyr Ala Phe Glu Trp Ser His Ala Asn Gly
 20 25 30

Ile Asp Asn Val Asp Ala Arg Glu Trp Ile Gly Ala Leu Ser Leu Arg
 35 40 45

Leu Pro Ala Ile Ala Thr Thr Met Tyr Leu Leu Phe Cys Leu Val Gly
 50 55 60

Pro Arg Leu Met Ala Lys Arg Glu Ala Phe Asp Pro Lys Gly Phe Met
 65 70 75 80

Leu Ala Tyr Asn Ala Tyr Gln Thr Ala Phe Asn Val Val Val Leu Gly
 85 90 95

Met Phe Ala Arg Glu Ile Ser Gly Leu Gly Gln Pro Val Trp Gly Ser
 100 105 110

Thr Met Pro Trp Ser Asp Arg Lys Ser Phe Lys Ile Leu Leu Gly Val
 115 120 125

Trp Leu His Tyr Asn Asn Lys Tyr Leu Glu Leu Leu Asp Thr Val Phe
 130 135 140

Met Val Ala Arg Lys Lys Thr Lys Gln Leu Ser Phe Leu His Val Tyr
 145 150 155 160

His His Ala Leu Leu Ile Trp Ala Trp Trp Leu Val Cys His Leu Met
 165 170 175

Ala Thr Asn Asp Cys Ile Asp Ala Tyr Phe Gly Ala Ala Cys Asn Ser
 180 185 190

Phe Ile His Ile Val Met Tyr Ser Tyr Tyr Leu Met Ser Ala Leu Gly
 195 200 205

Ile Arg Cys Pro Trp Lys Arg Tyr Ile Thr Gln Ala Gln Met Leu Gln
 210 215 220

Phe Val Ile Val Phe Ala His Ala Val Phe Val Leu Arg Gln Lys His
 225 230 235 240

Cys Pro Val Thr Leu Pro Trp Ala Gln Met Phe Val Met Thr Asn Met
 245 250 255

Leu Val Leu Phe Gly Asn Phe Tyr Leu Lys Ala Tyr Ser Asn Lys Ser
 260 265 270

Arg Gly Asp Gly Ala Ser Ser Val Lys Pro Ala Glu Thr Thr Arg Ala
 275 280 285

Pro Ser Val Arg Arg Thr Arg Ser Arg Lys Ile Asp
 290 295 300

<210> SEQ ID NO 21

<211> LENGTH: 1560

<212> TYPE: DNA

<213> ORGANISM: *Thraustochytrium ssp*

<400> SEQUENCE: 21

atgactgttg gatacgatga ggagatccca ttcgagcaag ttagggctca taacaagcca 60

gatgatgctt ggtgtgctat tcatggacac gtgtacgatg ttaccaagtt cgcttctggt 120

catccaggag gagatattat cttgctcgct gctgaaaagg aagctactgt gctctacgag 180

acctaccatg tttagaggagt gtctgatgct gtgctcagaa agtacagaat cggaaagttg 240

ccagatggac aaggaggagc taacgagaag gagaagagaa ccttgtctgg attgtcctct 300

gcttcttact acacctggaa ctccgatttc tacagagtga tgagggagag agttgtggct 360

agattgaagg agagagggaaa ggctagaaga ggaggatacg agttgtggat caaggctttc 420

ttgctccttg ttggattctg gtcctctctt tactggatgt gcaccctcga tccatctttc 480

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ggagctatct tggctgctat gtctttggga gtgttcgctg cttttgttg aacctgcac 540
caacatgatg gaaacctagg agctttcgct caatctagat gggtaacaa ggtggcagga 600
tggactttgg atatgatcgg agcttctgga atgacttggg agttccaaca tgtgttggga 660
catcacccat acactaactt gatcgaggag gagaacggat tgcaaaaggt gtccgaaaag 720
aagatggata ccaagtggc tgatcaagag tctgatccag atgtgttctc cacctacca 780
atgatgagat tgcacccatg gcatcagaag agatggtatc acaggttcca gcatatctac 840
ggaccattca tcttcggatt catgaccate aacaagggtg tgactcaaga tgttggagtg 900
gtgttgagaa agaggctctt ccaaatcgat gctgagtgca gatatgcttc cccaatgtac 960
gttgctaggt tctggatcat gaaggctttg accgtgttgt acatggttgc tctcccatgt 1020
tatatgcaag gaccatggca tggattgaag ctcttcgcta tgcctcattt cacttgcgga 1080
gaggttttgg ctaccatggt catcgtgaac cacattatcg agggagtgtc ttacgcttct 1140
aaggatgctg ttaagggaac tatggctcca ccaaagacta tgcattggagt gacccaatg 1200
aacaacacta gaaaggaggt tgaggctgag gcttctaagt ctggagctgt ggttaagtct 1260
gtgccattgg atgattgggc tgetgttcaa tgccaaacct ctgtgaactg gtctgttggga 1320
tcttggttct ggaaccattt ctctggagga ctcaaccatc aatcgagca tcatctcttc 1380
ccaggattgt ctcacgagac ctactaccac atccaagatg tggttcaatc tacctgtgct 1440
gagtacggag ttccatacca acatgagcca tctttgtgga ctgcttactg gaagatgctc 1500
gaacatttga gacaattggg aaacaggag actcacgagt cttggcaaag agctgcttga 1560

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<210> SEQ ID NO 22

<211> LENGTH: 519

<212> TYPE: PRT

<213> ORGANISM: *Thraustochytrium ssp*

<400> SEQUENCE: 22

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Met Thr Val Gly Tyr Asp Glu Glu Ile Pro Phe Glu Gln Val Arg Ala
1           5           10           15
His Asn Lys Pro Asp Asp Ala Trp Cys Ala Ile His Gly His Val Tyr
20           25           30
Asp Val Thr Lys Phe Ala Ser Val His Pro Gly Gly Asp Ile Ile Leu
35           40           45
Leu Ala Ala Gly Lys Glu Ala Thr Val Leu Tyr Glu Thr Tyr His Val
50           55           60
Arg Gly Val Ser Asp Ala Val Leu Arg Lys Tyr Arg Ile Gly Lys Leu
65           70           75           80
Pro Asp Gly Gln Gly Gly Ala Asn Glu Lys Glu Lys Arg Thr Leu Ser
85           90           95
Gly Leu Ser Ser Ala Ser Tyr Tyr Thr Trp Asn Ser Asp Phe Tyr Arg
100          105          110
Val Met Arg Glu Arg Val Val Ala Arg Leu Lys Glu Arg Gly Lys Ala
115          120          125
Arg Arg Gly Gly Tyr Glu Leu Trp Ile Lys Ala Phe Leu Leu Leu Val
130          135          140
Gly Phe Trp Ser Ser Leu Tyr Trp Met Cys Thr Leu Asp Pro Ser Phe
145          150          155          160
Gly Ala Ile Leu Ala Ala Met Ser Leu Gly Val Phe Ala Ala Phe Val
165          170          175
Gly Thr Cys Ile Gln His Asp Gly Asn His Gly Ala Phe Ala Gln Ser
180          185          190

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Arg Trp Val Asn Lys Val Ala Gly Trp Thr Leu Asp Met Ile Gly Ala
 195 200 205

Ser Gly Met Thr Trp Glu Phe Gln His Val Leu Gly His His Pro Tyr
 210 215 220

Thr Asn Leu Ile Glu Glu Glu Asn Gly Leu Gln Lys Val Ser Gly Lys
 225 230 235 240

Lys Met Asp Thr Lys Leu Ala Asp Gln Glu Ser Asp Pro Asp Val Phe
 245 250 255

Ser Thr Tyr Pro Met Met Arg Leu His Pro Trp His Gln Lys Arg Trp
 260 265 270

Tyr His Arg Phe Gln His Ile Tyr Gly Pro Phe Ile Phe Gly Phe Met
 275 280 285

Thr Ile Asn Lys Val Val Thr Gln Asp Val Gly Val Val Leu Arg Lys
 290 295 300

Arg Leu Phe Gln Ile Asp Ala Glu Cys Arg Tyr Ala Ser Pro Met Tyr
 305 310 315 320

Val Ala Arg Phe Trp Ile Met Lys Ala Leu Thr Val Leu Tyr Met Val
 325 330 335

Ala Leu Pro Cys Tyr Met Gln Gly Pro Trp His Gly Leu Lys Leu Phe
 340 345 350

Ala Ile Ala His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile
 355 360 365

Val Asn His Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val
 370 375 380

Lys Gly Thr Met Ala Pro Pro Lys Thr Met His Gly Val Thr Pro Met
 385 390 395 400

Asn Asn Thr Arg Lys Glu Val Glu Ala Glu Ala Ser Lys Ser Gly Ala
 405 410 415

Val Val Lys Ser Val Pro Leu Asp Asp Trp Ala Ala Val Gln Cys Gln
 420 425 430

Thr Ser Val Asn Trp Ser Val Gly Ser Trp Phe Trp Asn His Phe Ser
 435 440 445

Gly Gly Leu Asn His Gln Ile Glu His His Leu Phe Pro Gly Leu Ser
 450 455 460

His Glu Thr Tyr Tyr His Ile Gln Asp Val Val Gln Ser Thr Cys Ala
 465 470 475 480

Glu Tyr Gly Val Pro Tyr Gln His Glu Pro Ser Leu Trp Thr Ala Tyr
 485 490 495

Trp Lys Met Leu Glu His Leu Arg Gln Leu Gly Asn Glu Glu Thr His
 500 505 510

Glu Ser Trp Gln Arg Ala Ala
 515

<210> SEQ ID NO 23
 <211> LENGTH: 1491
 <212> TYPE: DNA
 <213> ORGANISM: *Saccharomyces cerevisiae*

<400> SEQUENCE: 23

gogcttaaga tagtgccaaa taccaaaagg cacaggtgca gacgatactt aaacaatagt 60

gctactacgc cacttegtga aagctaatat ctctttacct tgcatttggg catggttgc aa 120

acaggaggat caaaatacaa atggaatcaa gaatgctctt ctggtatgat actttttggt 180

tttcttttga gcccatgcgt acatttgagc tgttgaaca gtcaaaaata aaacggcaaa 240

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taaattgaac ttgaacacaa aagtaaacca aatccaagac caaacttcaa aagtatagtt 300
gggagcaaca aaaagattga aaataccttg attcaatggg acacggatct tagcgttacc 360
atgcttcttt tgatagtcac cccataggcg caatttaatg tggcaataaa agttccatag 420
ttctgaaagc acgaaaagac caattaatgt actcaagtca tccaatttca aatatgaata 480
gtatttgaat aacttagcat tcccaaaggg gaagccgtag ccaaagtaac cgaatgaaat 540
gagaccgctt agaaccagt aatggaaaca atttttgaac aggttgaaaa ttggcatagt 600
agctaaagag aattggtgaa caaataaggt ttcaaataat ctctttccat aatgtcctaa 660
aattaaaaaa tatgcaaccc tgtttaaaaa tggattatag tgggagctag cactgtgcca 720
tctatcaaca actgtgggaa tggtagatag ataataaaaa agggagttaa ccaagactgg 780
acccaatac tcacaaaaga agactaatct ccatgaaatt tggggaccca aatctttgat 840
gaagaattcc attgagtcac cagcctcttc ttgaaaaaac gattctgaaa taaccggaac 900
ttgtttagat tctcttttgt aggttaatct tatcctgtac ttgctgatat tgtggttatt 960
agcagagatt tttttcaaaa catcatctaa agtaggcttt ttggataagt caatttcagt 1020
gtcccttaac cctttagagc ggctttttat ggtgataggc attttcaaat taaattcaaa 1080
atatgtatct ctctcaataa gctcaattgg tttctagata gctaaataga atataatctt 1140
actgtcctcc gttctgtaaa attcacgctc ttagtccctt ttcataattc cttactttt 1200
tgcgtacaaa atgatatggt tattatattt ttcttttttt tttttcaaat tttttcttt 1260
tcttgaaaaa tttttcaaat tggaaagctc atctctcttg aatgtataat actttcttcc 1320
tctaactttc aaaaagtttt acatagccaa gaagttttcc ttacatcggg atactactgt 1380
tatataagtt attcttcgag aaacaattag atatcattca tcggataaat ctaagttgcc 1440
cattgctttc aataactccg atcaaatata ctcaaatcaa ctaaaacagt a 1491

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<210> SEQ ID NO 24

<211> LENGTH: 310

<212> TYPE: PRT

<213> ORGANISM: *Saccharomyces cerevisiae*

<400> SEQUENCE: 24

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Met Pro Ile Thr Ile Lys Ser Arg Ser Lys Gly Leu Arg Asp Thr Glu
1           5           10           15
Ile Asp Leu Ser Lys Lys Pro Thr Leu Asp Asp Val Leu Lys Lys Ile
20           25           30
Ser Ala Asn Asn His Asn Ile Ser Lys Tyr Arg Ile Arg Leu Thr Tyr
35           40           45
Lys Lys Glu Ser Lys Gln Val Pro Val Ile Ser Glu Ser Phe Phe Gln
50           55           60
Glu Glu Ala Asp Asp Ser Met Glu Phe Phe Ile Lys Asp Leu Gly Pro
65           70           75           80
Gln Ile Ser Trp Arg Leu Val Phe Phe Cys Glu Tyr Leu Gly Pro Val
85           90           95
Leu Val His Ser Leu Phe Tyr Tyr Leu Ser Thr Ile Pro Thr Val Val
100          105          110
Asp Arg Trp His Ser Ala Ser Ser Asp Tyr Asn Pro Phe Leu Asn Arg
115          120          125
Val Ala Tyr Phe Leu Ile Leu Gly His Tyr Gly Lys Arg Leu Phe Glu
130          135          140
Thr Leu Phe Val His Gln Phe Ser Leu Ala Thr Met Pro Ile Phe Asn
145          150          155          160

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Leu Phe Lys Asn Cys Phe His Tyr Trp Val Leu Ser Gly Leu Ile Ser
 165 170 175

Phe Gly Tyr Phe Gly Tyr Gly Phe Pro Phe Gly Asn Ala Lys Leu Phe
 180 185 190

Lys Tyr Tyr Ser Tyr Leu Lys Leu Asp Asp Leu Ser Thr Leu Ile Gly
 195 200 205

Leu Phe Val Leu Ser Glu Leu Trp Asn Phe Tyr Cys His Ile Lys Leu
 210 215 220

Arg Leu Trp Gly Asp Tyr Gln Lys Lys His Gly Asn Ala Lys Ile Arg
 225 230 235 240

Val Pro Leu Asn Gln Gly Ile Phe Asn Leu Phe Val Ala Pro Asn Tyr
 245 250 255

Thr Phe Glu Val Trp Ser Trp Ile Trp Phe Thr Phe Val Phe Lys Phe
 260 265 270

Asn Leu Phe Ala Val Leu Phe Leu Thr Val Ser Thr Ala Gln Met Tyr
 275 280 285

Ala Trp Ala Gln Lys Lys Asn Lys Lys Tyr His Thr Arg Arg Ala Phe
 290 295 300

Leu Ile Pro Phe Val Phe
 305 310

<210> SEQ ID NO 25
 <211> LENGTH: 1042
 <212> TYPE: DNA
 <213> ORGANISM: *Saccharomyces cerevisiae*

<400> SEQUENCE: 25

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gaaataatac taattaaata attctaataa ttctaataatt aataacaata ataataagaa    60
taataaattat acaataaacac atgtatttcc taactcaciaa tcgtttggac tacatatgct    120
gtctagtgcc ttattgcgac tttgcccgtt tgataactta cttcgattgt ttagtattca    180
aaaaggaaaa aggcggttttc tttttcgtgt acttttttttc gaaattctgt tgaattttatt    240
cgaactcaga attggtccat caagagcadc caaaatacaa aataactcat catcacaciaa    300
gaagaagcac aactccaagc aatttttaca atatgtcaaa aaaacttgcg tcaccattgt    360
ccttcttacc cctttataat ttgctttctg ctggttggtg gtcttatttg ctttacttgg    420
tcatctcctt gtaccacaaa gttggacagc cagcattcct ctaccaaact aaaaatgctg    480
ctacccttgt tcaatgtggt gctataatcg agatcataaa ctcatTTTTA ggagttgtac    540
gttccccatt gctgaccact gttgcacaag tgtcttcaag actactagtt gtectcgcca    600
tcttccaatt gttgccaaa acaagtgggt ttcaatcagt tgtttacata tcattattac    660
tggcatggtc tataactgag atcgtcagat acttgtatta ttttttcatg ttggatttca    720
agaatggcgc accaaaagat ttaattctat taagatataa tttggtctgg attttgtacc    780
cactggtgt tgccagcgaa ctacgcatta tttactgtgc tttaatgca gctgaatctc    840
agtattcttt actttacaaa agaattttaa tagcggccat gctcgcttat atcccaggct    900
tccaatgct cttcctacac atggtagcac agagaaagaa agtcatgaaa agtttaagat    960
cctctttcgg gaagaaacta atttgaattc tttagataaa ttcttctgta ttttttataa   1020
gaatattagc cttcacattg aa                                           1042
    
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<210> SEQ ID NO 26
 <211> LENGTH: 217
 <212> TYPE: PRT
 <213> ORGANISM: *Saccharomyces cerevisiae*

-continued

<400> SEQUENCE: 26

Met Ser Lys Lys Leu Ala Ser Pro Leu Ser Phe Leu Pro Leu Tyr Asn
 1 5 10 15
 Leu Leu Ser Ala Val Gly Trp Ser Tyr Leu Leu Tyr Leu Val Ile Ser
 20 25 30
 Leu Tyr Pro Lys Val Gly Gln Pro Ala Phe Phe Tyr Gln Thr Lys Asn
 35 40 45
 Val Ala Thr Leu Val Gln Cys Gly Ala Ile Ile Glu Ile Ile Asn Ser
 50 55 60
 Phe Leu Gly Val Val Arg Ser Pro Leu Leu Thr Thr Val Ala Gln Val
 65 70 75 80
 Ser Ser Arg Leu Leu Val Val Leu Gly Ile Phe Gln Leu Leu Pro Asn
 85 90 95
 Thr Ser Gly Val Gln Ser Val Val Tyr Ile Ser Leu Leu Leu Ala Trp
 100 105 110
 Ser Ile Thr Glu Ile Val Arg Tyr Leu Tyr Tyr Phe Phe Met Leu Val
 115 120 125
 Phe Lys Asn Gly Ala Pro Lys Ile Leu Ile Leu Leu Arg Tyr Asn Leu
 130 135 140
 Phe Trp Ile Leu Tyr Pro Thr Gly Val Ala Ser Glu Leu Arg Ile Ile
 145 150 155 160
 Tyr Cys Ala Leu Asn Ala Ala Glu Ser Gln Tyr Ser Leu Leu Tyr Lys
 165 170 175
 Arg Ile Leu Ile Ala Ala Met Leu Ala Tyr Ile Pro Gly Phe Pro Met
 180 185 190
 Leu Phe Leu His Met Val Ala Gln Arg Lys Lys Val Met Lys Ser Leu
 195 200 205
 Arg Ser Ser Phe Gly Lys Lys Leu Ile
 210 215

The invention claimed is:

1. A polynucleotide comprising an expression control sequence operatively linked to a heterologous nucleic acid sequence selected from the group consisting of:

- a) the nucleic acid sequence of SEQ ID NO 1 or 3;
- b) a nucleic acid sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2 or 4;
- c) a nucleic acid sequence having at least 50% sequence identity to the nucleic acid sequence of a) or b), wherein said nucleic acid sequence encodes a polypeptide having fatty acid dehydratase/enoyl-CoA reductase (nECR) activity; and
- d) a nucleic acid sequence encoding a polypeptide having nECR activity and comprising an amino acid sequence having at least 50% sequence identity to the amino acid sequence of SEQ ID NO 2 or 4.

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

3. A vector comprising the polynucleotide of claim 1.

4. A host cell comprising the polynucleotide of claim 1.

5. A method for the manufacture of a polypeptide comprising:

- a) cultivating a host cell comprising the polynucleotide of claim 1 under conditions which allow for the production of a polypeptide encoded by said nucleic acid sequence; and
- b) obtaining the polypeptide from the host cell of step a).

6. A non-human transgenic organism comprising the polynucleotide of claim 1, wherein said organism is a microorganism, yeast, or plant.

7. A plant, plant part, or plant seed comprising the polynucleotide of claim 1.

8. A method for the manufacture of a polyunsaturated fatty acid comprising:

- a) cultivating the host cell of claim 4 under conditions which allow for the production of polyunsaturated fatty acids in said host cell; and
- b) obtaining said polyunsaturated fatty acids from said host cell.

9. The method of claim 8, wherein said polyunsaturated fatty acid is arachidonic acid (ARA), eicosapentaenoic acid (EPA), or docosahexaenoic acid (DHA).

10. A method for the manufacture of an oil, lipid, or fatty acid composition comprising:

- a) cultivating the host cell of claim 4 under conditions which allow for the production of polyunsaturated fatty acids in said host cell;
- b) obtaining said polyunsaturated fatty acids from said host cell; and
- c) formulating the polyunsaturated fatty acid as an oil, lipid, or fatty acid composition.

11. The method of claim 10, wherein said oil, lipid, or fatty acid composition is used for feed, foodstuffs, cosmetics, or medicaments.

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12. A method for the manufacture of a polyunsaturated fatty acid comprising:

- a) cultivating the non-human transgenic organism of claim 6 under conditions which allow for the production of polyunsaturated fatty acids in said organism; and
- b) obtaining said polyunsaturated fatty acids from said non-human transgenic organism.

13. The method of claim 12, wherein the polyunsaturated fatty acid is arachidonic acid (ARA), eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA).

14. A method for the manufacture of an oil, lipid, or fatty acid composition comprising:

- a) cultivating the non-human transgenic organism of claim 6 under conditions which allow for the production of polyunsaturated fatty acids in said organism;
- b) obtaining said polyunsaturated fatty acids from said non-human transgenic organism;
- c) formulating the polyunsaturated fatty acid as an oil, lipid, or fatty acid composition.

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

16. A polynucleotide comprising an expression control sequence operatively linked to a heterologous nucleic acid sequence selected from the group consisting of:

- a) the nucleic acid sequence of SEQ ID NO: 1 or 3;
- b) a nucleic acid sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2 or 4; and

- c) a nucleic acid sequence encoding a polypeptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 2 or 4 and having nECR activity.

17. The polynucleotide of claim 16, wherein said nucleic acid sequence encodes a polypeptide having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 2 or 4 and having nECR activity.

* * * * *