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(54) Title: Δ4-DESATURASE GENES AND USES THEREOF

FATTY ACID BIOSYNTHETIC PATHWAY
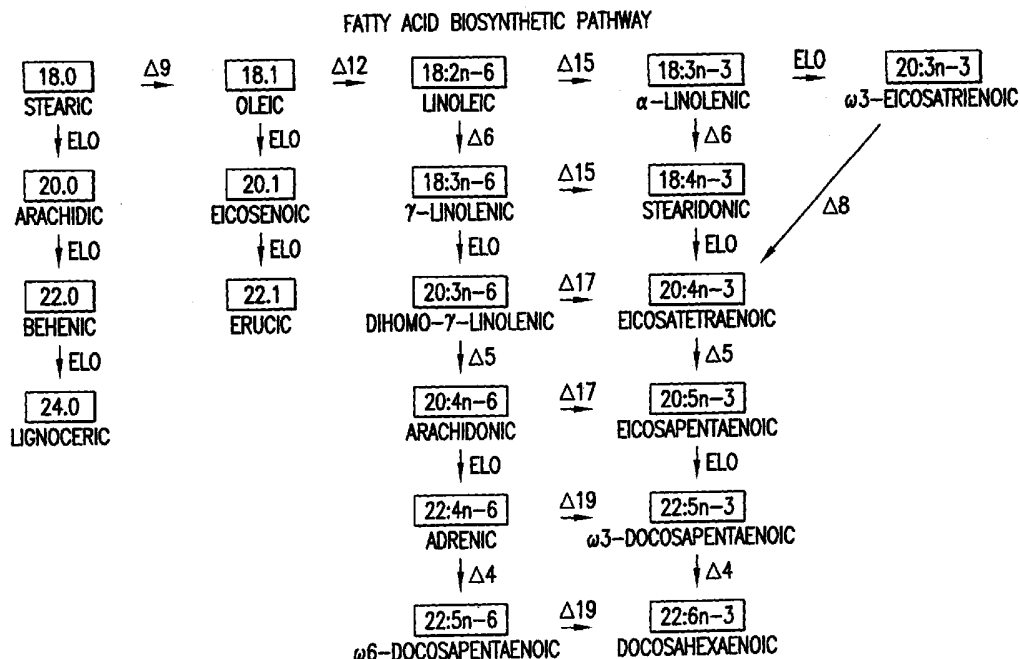
(57) **Abstract:** The subject invention relates to the identification of genes involved in the desaturation of polyunsaturated fatty acids at carbon 4 (i.e., "Δ4-desaturase"). In particular, Δ4-desaturase may be utilized, for example, in the conversion of adrenic acid to ω6-docosapentaenoic acid and in the conversion of ω3-docosapentaenoic acid to docosahexaenoic acid. The polyunsaturated fatty acids produced by use of the enzyme may be added to pharmaceutical compositions, nutritional compositions, animal feeds, as well as other products such as cosmetics.

Δ4-DESATURASE GENES AND USES THEREOF

BACKGROUND OF THE INVENTION

Technical Field

The subject invention relates to the identification and isolation of genes that encode enzymes (e.g., *Thraustochytrium aureum* Δ4-desaturase, *Schizochytrium aggregatum* Δ4-desaturase and *Isochrysis galbana* Δ4-desaturase) involved in the synthesis of polyunsaturated fatty acids and to uses thereof. In particular, Δ4-desaturase catalyzes the conversion of, for example, adrenic acid (22:4n-6) to ω6-docosapentaenoic acid (22:5n-6) and the conversion of ω3-docosapentaenoic acid (22:5n-3) to docosahexaenoic acid (22:6n-3). The converted products may then be utilized as substrates in the production of other polyunsaturated fatty acids (PUFAs). The product or other polyunsaturated fatty acids may be added to pharmaceutical compositions, nutritional composition, animal feeds as well as other products such as cosmetics.

Background Information

Desaturases are critical in the production of long-chain polyunsaturated fatty acids that have many important functions. For example, polyunsaturated fatty acids (PUFAs) are important components of the plasma membrane of a cell, where they are found in the form of phospholipids. They also serve as precursors to mammalian prostacyclins, eicosanoids, leukotrienes and prostaglandins.

Additionally, PUFAs are necessary for the proper development of the developing infant brain as well as for tissue formation and repair. In view of the biological significance of PUFAs, attempts are being made to produce them, as well as intermediates leading to their production, in an efficient manner.

A number of enzymes, most notably desaturase and

elongases, are involved in PUFA biosynthesis (see Figure 1).

For example, elongase (elo) catalyzes the conversion of γ-

linolenic acid (GLA) to dihomo-γ-linolenic acid (DGLA) and of

5   stearidonic acid (18:4n-3) to (n-3)-eicosatetraenoic acid

.   (20:4n-3). Linoleic acid (LA, 18:2n-9,12 or 18:2n-6) is

produced from oleic acid (18:1-Δ9) by a Δ12-desaturase. GLA

(18:3n-6,9,12) is produced from linoleic acid by a Δ6-

desaturase.

10   It must be noted that animals cannot desaturate beyond

the Δ9 position and therefore cannot convert oleic acid into

linoleic acid. Likewise, γ-linolenic acid (ALA, 18:3n-

9,12,15) cannot be synthesized by mammals. However, γ-

linolenic acid can be converted to stearidonic acid (STA,

15   18:4n-6,9,12,15) by a Δ6-desaturase (see PCT publication WO

96/13591 and The FASEB Journal, Abstracts, Part I, Abstract

3093, page A532 (Experimental Biology 98, San Francisco, CA,

April 18-22, 1998); see also U.S. Patent No. 5,552,306),

followed by elongation to (n-3)-eicosatetraenoic acid (20:4n-

20   8,11,14,17) in mammals and algae. This polyunsaturated fatty

acid (i.e., 20:4n-8,11,14,17) can then be converted to

eicosapentaenoic acid (EPA, 20:5n-5,8,11,14,17) by a Δ5-

desaturase. EPA can then, in turn, be converted to ω3-

docosapentaenoic acid (22:5n-3) by an elongase. Isolation of

25   an enzyme or its encoding gene, responsible for conversion of

ω3-docosapentaenoic acid to docosahexaenoic acid (22:6n-3) has

never been reported. Two pathways for this conversion have

been proposed (see Figure 1 and Sprecher, H., Curr. Opin.

Clin. Nutr. Metab. Care, Vol.2, p. 135-138, 1999). One of

30   them involves a single enzyme, a Δ4-desaturase such as that of

the present invention. In the n-6 pathway, dietary linoleic

acid may be converted to adrenic acid through a series of

desaturation and elongation steps in mammals (see Figure 1).

Production of ω6-docosapentaenoic acid from adrenic acid is

postulated to be mediated by the Δ6-desaturase discussed above.

Other eukaryotes, including fungi and plants, have enzymes which desaturate at carbon 12 (see PCT publication WO 94/11516 and U.S. Patent No. 5,443,974) and carbon 15 (see PCT publication WO 93/11245). The major polyunsaturated fatty acids of animals therefore are either derived from diet and/or from desaturation and elongation of linoleic acid or γ-linolenic acid. In view of these difficulties, it is of significant interest to isolate genes involved in PUFA synthesis from species that naturally produce these fatty acids and to express these genes in a microbial, plant, or animal system which can be altered to provide production of commercial quantities of one or more PUFAs.

In view of the above discussion, there is a definite need for the Δ4-desaturase enzyme, the respective genes encoding this enzyme, as well as recombinant methods of producing this enzyme. Additionally, a need exists for oils containing levels of PUFAs beyond those naturally present as well as those enriched in novel PUFAs. Such oils can only be made by isolation and expression of the Δ4-desaturase gene(s).

All U.S. patents and publications referred to herein are hereby incorporated in their entirety by reference.

SUMMARY OF THE INVENTION

The present invention includes an isolated nucleotide sequence or fragment thereof comprising or complementary to a nucleotide sequence encoding a polypeptide having desaturase activity. The amino acid sequence of the polypeptide has at least 50% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID NO:46 and SEQ ID NO:55. Also, in particular, the present invention encompasses an isolated nucleotide sequence or fragment thereof comprising or complementary to a nucleotide sequence encoding a

polypeptide having desaturase activity, wherein the amino acid
sequence of said polypeptide has at least 30% identity to the
amino acid sequence of SEQ ID NO:55.

Additionally, the present invention encompasses an
5   isolated nucleotide sequence or fragment thereof comprising or
·   complementary to a nucleotide sequence having at least 50%
sequence identity to a nucleotide sequence selected from the
group consisting of SEQ ID NO:14, SEQ ID NO:15 and SEQ ID
NO:16, SEQ ID NO:17, SEQ ID NO:36, SEQ ID NO:45 and SEQ ID
10   NO:54. In particular, the present invention includes an
isolated nucleotide sequence or fragment thereof comprising or
complementary to a nucleotide sequence having at least 40%
identity to the nucleotide sequence of SEQ ID NO:54.

Each of the sequences described above encodes a
15   functionally active desaturase that utilizes a monounsaturated
or polyunsaturated fatty acid as a substrate. The nucleotide
sequences may be derived for example, from a fungus or an
algae. In particular, when the nucleotide sequence comprises
SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17 or SEQ
20   ID NO:45, may be derived, for example, from the fungus
Thraustochytrium aureum. The sequence comprising SEQ ID NO:36
may be derived, for example, from the fungus *Schizochytrium
aggregatum*. The sequence comprising SEQ ID NO:54 may be
derived, for example, from the algae *Isochrysis galbana*. The
25   present invention also includes purified protein and fragments
thereof encoded by the above-referenced nucleotide sequences.

In particuar, the present invention also includes a
purified polypeptide which desaturates polyunsaturated fatty
acids at carbon 4 and has an amino acid sequence having at
30   least 50% identity to an amino acid sequence selected from the
group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20,
SEQ ID NO:21, SEQ ID NO:37, SEQ ID NO:46 and SEQ ID NO:55. In
particular, the present invention also includes a purified
polypeptide which desaturates polyunsaturated fatty acids at

carbon 4 and has an amino acid sequence having at least 30%
identity to the amino acid sequence of SEQ ID NO:55.

　　　　　Additionally, the present invention includes' a
Method of producing a desaturase comprising the steps of:
5　　isolating a nucleotide sequence comprising or complementary to
a nucleotide sequence encoding a polypeptide having an amino
acid sequence having at least 50% identity to an amino acid
sequence selected from the group consisting of SEQ ID NO:18,
SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID
10　NO:46 and SEQ ID NO:55 (or at least 30% identity to the amino
acid sequence of SEQ ID NO:55) or having at least 50% sequence
identity to a nucleotide sequence selected from the group
consisting of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID
NO:17, SEQ ID NO:36, SEQ ID NO:45 and SEQ ID NO:54 (or having,
15　in particular, at least 40% sequence identity to SEQ ID
NO:54); constructing a vector comprising: i) the isolated
nucleotide sequence operably linked to ii) a promoter; and
introducing said vector into a host cell for a time and under
conditions sufficient for expression of the desaturase. The
20　host cell may be, for example, a eukaryotic cell or a
prokaryotic cell. In particular, the prokaryotic cell may be,
for example, E. coli, cyanobacteria or B. subtilis. The
eukaryotic cell may be, for example, a mammalian cell, an
insect cell, a plant cell or a fungal cell (e.g., a yeast cell
25　such as Saccharomyces cerevisiae, Saccharomyces
carlsbergensis, Candida spp., Lipomyces starkey, Yarrowia
lipolytica, Kluyveromyces spp., Hansenula spp., Trichoderma
spp. or Pichia spp.).

　　　　　Moreover, the present invention also includes a vector
30　comprising: an isolated nucleotide sequence comprising or
complementary to a nucleotide sequence encoding a polypeptide
having an amino acid sequence having at least 50% amino acid
identity to an amino acid sequence selected from the group
consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID
35　NO:21, SEQ ID NO:37, SEQ ID NO:46 and SEQ ID NO:55 (or, in
particular, at least 30% amino acid identity to SEQ ID NO:55)

or having at least 50% sequence identity to a nucleotide
sequence selected from the group consisting of SEQ ID NO:14,
SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:36, SEQ ID
NO:45 and SEQ ID NO:54 (or, in particular, at least 40%
identity to SEQ ID NO:54), operably linked to a promoter. The
invention also includes a host cell comprising this vector.
The host cell may be, for example, a eukaryotic cell or a
prokaryotic cell. Suitable eukaryotic cells and prokaryotic
cells are as defined above.

Moreover, the present invention also includes a plant
cell, plant or plant tissue comprising the above vector,
wherein expression of the nucleotide sequence of the vector
results in production of a polyunsaturated fatty acid by the
plant cell, plant or plant tissue. The polyunsaturated fatty
acid may be, for example, selected from the group consisting
of ω6-docosapentaenoic acid or docosahexaenoic acid. The
invention also includes one or more plant oils or acids
expressed by the above plant cell, plant or plant tissue.

Additionally, the present invention also encompasses a
transgenic plant comprising the above vector, wherein
expression of the nucleotide sequence of the vector results in
production of a polyunsaturated fatty acid in seeds of the
transgenic plant.

The present invention also includes a method ("first
method") for producing a polyunsaturated fatty acid comprising
the steps of: isolating a nucleotide sequence comprising or
complementary to a nucleotide sequence encoding a polypeptide
having an amino acid sequence having at least 50% amino acid
sequence identity to an amino acid sequence selected from the
group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20,
SEQ ID NO:21, SEQ ID NO:37, SEQ ID NO:46 and SEQ ID NO:55
(and, in particular, at least 30% amino acid sequence identity
to SEQ ID NO:55) or having at least 50% sequence identity to a
nucleotide sequence selected from the group consisting of SEQ
ID NO:14, SEQ ID NO:15, SEQ ID NO:16 and SEQ ID NO:17, SEQ ID

NO:36, SEQ ID NO:45, and SEQ ID NO:54 (and, in particular, at least 40% with respect to SEQ ID NO:54); constructing a vector comprising the isolated nucleotide sequence; introducing the vector into a host cell for a time and under conditions

5   sufficient for expression of Δ4-desaturase; and exposing the expressed Δ4-desaturase to a substrate polyunsaturated fatty acid in order to convert the substrate to a product polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be, for example, adrenic acid or ω3-

10 docospentaenoic acid, and the product polyunsaturated fatty acid may be, for example, ω6-docosapentaenoic acid or docosahexaenoic acid, respectively. This method may further comprise the step of exposing the product polyunsaturated fatty acid to another enzyme (e.g., a Δ4-desaturase, an

15 elongase or another desaturase) in order to convert the product polyunsaturated fatty acid to another polyunsaturated fatty acid (i.e., "second" method). In this method containing the additional step (i.e., "second" method), the product polyunsaturated fatty acid may be, for example, ω6-

20 docosapentaenoic acid, and the "another" polyunsaturated fatty acid may be docosahexaenoic acid.

Also, the present invention includes a method of producing a polyunsaturated fatty acid comprising the steps of: exposing a substrate polyunsaturated fatty acid to one or

25 more enzymes selected from the group consisting of a desaturase and an elongase in order to convert the substrate to a product polyunsaturated fatty acid; and exposing the product polyunsaturated fatty acid to a Δ4-desaturase comprising an amino acid sequence selected

30 from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID NO:46 and SEQ ID NO:55, in order to convert the product polyunsaturated fatty acid to a final product polyunsaturated fatty acid.

For example, a substrate polyunsaturated fatty acid

35 (e.g., eicosapentaenoic acid) may be exposed to an elongase or

desaturase (e.g., MELO4 or other elongases or desaturases of
significance in the biosynthetic pathway) in order to convert
the substrate to a product polyunsaturated fatty acid (e.g.,
ω3-docosapentaenoic acid). The product polyunsaturated fatty
acid may then be converted to a "final" product
polyunsaturated fatty acid (e.g., docosahexaenoic acid) by
exposure to the Δ4-desaturase of the present invention (see
Figure 1). Thus, the Δ4-desaturase is utilized in the last
step of the method in order to create the "final" desired
product. As another example, one may expose linoleic acid to
a Δ6-desaturase in order to create γ-linolenic acid (GLA), and
then expose the GLA to an elongase and then to a Δ5-desaturase
in order to create arachidonic acid (AA). The AA may then be
exposed to an elongase in order to convert it to adrenic acid.
Finally, the adrenic acid may be exposed to Δ4-desaturase in
order to convert it to ω6-docosapentaenoic acid (see Figure
1). Thus, the method involves the utilization of a linoleic
acid substrate and a series of desaturase and elongase
enzymes, in addition to the Δ4-desaturase, in order to arrive
at the final product. By use of a similar method, one may
also convert the substrate PUFA, γ-linolenic acid to
docosoahexaenoic acid. Again, various desaturases and
elongase are used to ultimately arrive at ω3-docosapentaenoic
acid which is then exposed to one or more of the Δ4-
desaturases of the present invention in order to convert it to
docosahexaenoic acid. (Possible substrates include those
shown in Figure 1, for example, linoleic acid, γ-linolenic
acid, stearidonic acid, arachidonic acid, dihomo-γ-linolenic
acid, adrenic acid, eicosapentaenoic acid and eicosatetraenoic
acid.)

       The present invention also encompasses a composition
comprising at least one polyunsaturated fatty acid selected
from the group consisting of the "product" polyunsaturated
fatty acid produced according to the methods described above
and the "another" polyunsaturated fatty acid produced

according to the methods described above. The product
polyunsaturated fatty acid may be, for example, ω6-
docosapentaenoic acid or docosahexaenoic acid. The another
polyunsaturated fatty acid may be, for example,
5   docosahexaenoic acid.

    Additionally, the present invention encompasses a method
of preventing or treating a condition caused by insufficient
intake of polyunsaturated fatty acids comprising administering
to the patient the composition above in an amount sufficient
10 to effect prevention or treatment.

                    BRIEF DESCRIPTION OF THE DRAWINGS

        Figure 1 illustrates the fatty acid biosynthetic pathway
    and the role of Δ4-desaturase in this pathway.

15   Figure 2 illustrates an amino acid comparison of Δ4-
    desaturases produced by four different plasmids (SEQ ID NO:18,
    SEQ ID NO:19, SEQ ID NO:20 and SEQ ID NO:21).

        Figure 3 illustrates the nucleotide sequence encoding Δ4-
    desaturase of Thraustochytrium aureum (ATCC 34304) from
20 plasmid pRTA5 (SEQ ID NO:14).

        Figure 4 illustrates the nucleotide sequence encoding Δ4-
    desaturase of Thraustochytrium aureum (ATCC 34304) from
    plasmid pRTA6 (SEQ ID NO:15).

        Figure 5 illustrates the nucleotide sequence encoding Δ4-
25 desaturase of Thraustochytrium aureum (ATCC 34304) from
    plasmid pRTA7 (SEQ ID NO:16).

        Figure 6 illustrates the nucleotide sequence encoding
    encoding Δ4-desaturase of Thraustochytrium aureum (ATCC 34304)
    from plasmid pRTA8 (SEQ ID NO:17).

30   Figure 7 illustrates the amino acid sequence of Δ4-
    desaturase of Thraustochytrium aureum (ATCC 34304) from
    plasmid pRTA5 (SEQ ID NO:18).

                                    9

Figure 8 illustrates the amino acid sequence of Δ4-desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA6 (SEQ ID NO:19).

Figure 9 illustrates the amino acid sequence of Δ4-desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA7 (SEQ ID NO:20).

Figure 10 illustrates the amino acid sequence of Δ4-desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA8 (SEQ ID NO:21).

Figure 11 illustrates the nucleotide and amino acid sequences described herein.

Figure 12 illustrates the amino acid sequence encoded by the elongase gene MELO4 from a mouse.

Figure 13 illustrates the DNA sequence of the putative Δ4-desaturase ssa.con (SEQ ID NO:24) generated from clones saa9 and saa5 from *S. aggregatum* (ATCC 28209) (see Example VI).

Figure 14 illustrates the amino acid sequence (SEQ ID NO:25) of the putative Δ4-desaturase encoded by the ssa.con DNA sequence from *S. aggregatum* (ATCC 28209) (see Example VI).

Figure 15 illustrates the alignment of the amino acids derived from the translation of the open reading frames of ssa.con DNA from *S. aggregatum* (ATCC 28209) (SEQ ID NO:25) and pRTA7 (SEQ ID NO:68) (see Example VI).

Figure 16 illustrates the DNA sequence of the Δ4-desaturase from pRSA1 (SEQ ID NO:36) *S. aggregatum* (ATCC 28209) (see Example VII).

Figure 17 illustrates the amino acid sequence (SEQ ID NO:37) of the Δ4-desaturase encoded by the pRSA1 DNA sequence from *S. aggregatum* (ATCC 28209) (see Example VII).

Figure 18 illustrates the DNA sequence of the Δ4-desaturase from pRTA11 (SEQ ID NO:45) *T. aureum* (BICC 7091) (see Example VII).

Figure 19 illustrates the amino acid sequence (SEQ ID

NO:46) of the putative Δ4-desaturase encoded by the pRTA11 DNA

sequence from *T. aureum* (BICC 7091) (see Example VII).

Figure 20 illustrates the DNA sequence of the Δ4-

5   desaturase from *Isochrysis galbana* (CCMP1323)(SEQ ID NO: 54)

present in clone pRIG6 (see Example IX).

Figure 21 illustrates the amino acid sequence (SEQ ID

NO:55) of the Δ4-desaturase encoded by the pRIG6 DNA sequence

from *Isochrysis galbana* (CCMP1323) (see Example IX).

10   Figure 22 illustrates the percent identity between the

novel Δ4-desaturase from *I. galbana* (CCMP 1323) (SEQ ID NO:69)

and the Δ4-desaturase from *Thraustochytrium aureum* (ATCC

34304) (SEQ ID NO:70).

15   DETAILED DESCRIPTION OF THE INVENTION

The subject invention relates to the nucleotide and

translated amino acid sequences of the Δ4-desaturase gene

derived from the fungus *Thraustochytrium aureum* (BICC 7091),

the fungus *Schizochytrium aggregatum*, and the algae *Isochrysis*

20   *galbana*. Furthermore, the subject invention also includes

uses of these genes and of the enzymes encoded by these genes.

For example, the genes and corresponding enzymes may be used

in the production of polyunsaturated fatty acids such as, for

instance, ω6-docosapentaenoic acid and/or docosahexaenoic acid

25   which may be added to pharmaceutical compositions, nutritional

compositions and to other valuable products.

The Δ4-Desaturase Genes and Enzymes Encoded Thereby

As noted above, the enzymes encoded by the Δ4-desaturase

30   genes of the present invention are essential in the production

of highly unsaturated polyunsaturated fatty acids having a

length greater than 22 carbons. The nucleotide sequences of

the isolated *Thraustochytrium aureum* Δ4-desaturase genes,

which differed based upon the plasmid created (see Example

III), are shown in Figures 3-6, and the amino acid sequences
of the corresponding purified proteins are shown in Figure 7-
10. An additional, isolated *T. aureum* nucleotide sequence is
shown in Figure 18 (see Example VII), and the encoded amino
acid sequence is shown in Figure 19. The nucleotide sequences
of the isolated *Schizochytrium aggregatum* Δ4-desaturase
sequences are shown in Figures 13 and 16, and the encoded
amino acid sequences are shown in Figures 14 and 17,
respectively. Additionally, the nucleotide sequences of the
isolated *Isochrysis galbana* Δ4-desaturase sequence is shown in
Figure 20, and the amino acid sequence encoded by the
nucleotide sequence is shown in Figure 21.

It should be noted that the present invention also
encompasses isolated nucleotide sequences (and the
corresponding encoded proteins) having sequences comprising,
corresponding to, identical to, or complementary to at least
about 50%, preferably at least about 60%, and more preferably
at least about 70%, even more preferably at least about 80%,
and most preferably at least about 90% sequence identity to
SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16 or SEQ ID NO:17
(i.e., the nucleotide sequences of the Δ4-desaturase gene of
Thraustochytrium aureum (ATCC 34304)), to SEQ ID NO:36 (i.e.,
the nucleotide sequence of the Δ4-desaturase gene of
Schizochytrium aggregatum (ATCC 28209) or to SEQ ID NO:45
(i.e., the nucleotide sequence of the Δ4-desaturase gene of
Thraustochytrium aureum (BICC 7091)) or to SEQ ID NO:54 (i.e.,
the nucleotide sequence of the Δ4-desaturase gene of
Isochrysis galabana), all described herein. With respect to
the *I. galbana* sequence, in particular, the present invention
also encompasses nucleotide sequences (and the corresponding
encoded proteins) having sequences comprising, corresponding

                                    12

to, identical to, or complementary to at least 40%, more
preferably at least 60%, even more preferably at least 80%,
and most preferably at least 90% of the nucleotide sequence of
SEQ ID NO:54. (All integers between 40% and 100% are also

5   considered to be within the scope of the present invention
with respect to percent identity.) Such sequences may be
derived from any source, either isolated from a natural
source, or produced via a semi-synthetic route, or synthesized
de novo. In particular, such sequences may be isolated or

10 derived from sources other than described in the examples
(e.g., bacteria, fungus, algae, C. elegans, mouse or human).

        Furthermore, the present invention also encompasses
fragments and derivatives of the nucleotide sequences of the
present invention (i.e., SEQ ID NO:14, SEQ ID NO:15, SEQ ID

15 NO:16, SEQ ID NO:17 ,SEQ ID NO:36, SEQ ID NO:45 or SEQ ID
NO:54), as well as of the sequences derived from other
sources, and having the above-described complementarity,
identity or correspondence. Functional equivalents of the
above full length sequences and fragments (i.e., sequences

20 having Δ4-desaturase activity, as appropriate) are also
encompassed by the present invention.

        For purposes of the present invention, a "fragment" is of
a nucleotide sequence is defined as a contiguous sequence of
approximately at least 6, preferably at least about 8, more

25 preferably at least about 10 nucleotides, and even more
preferably at least about 15 nucleotides corresponding to a
region of the specified nucleotide sequence.

        The invention also includes a purified polypeptide which
desaturates polyunsaturated fatty acids at the carbon 4

30 position and has at least about 50% amino acid similarity or
identity, preferably at least about 60% amino acid similarity
or identity, more preferably at least about 70% amino acid
similarity or identity, even more preferably at least about
80% amino acid similarity or identity and most preferably at

35 least 90% amino acid similarity or identity to the amino acid

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sequences (i.e., SEQ ID NO:18 (shown in Figure 7), SEQ ID
NO:19 (shown in Figure 8), SEQ ID NO:20 (shown in Figure 9),
SEQ ID NO:21 (shown in Figure 10), SEQ ID NO:37 (shown in
Figure 17), SEQ ID NO:46 (shown in Figure 19) and SEQ ID NO:55

5   (shown in Figure 21) of the above-noted proteins which are, in
turn, encoded by the above-described nucleotide sequences. In
particular, with respect to the amino acid sequence of the *I.
galbana* Δ4-desaturase, the present invention encompasses
includes a purified polypeptide which desaturates

10   polyunsaturated fatty acids at the carbon 4 position and has
at least about 30% amino acid similarity or identity,
preferably at least about 50% amino acid similarity or
identity, more preferably at least about 70% amino acid
similarity or identity and most preferably at least about 90%

15   amino acid similarity or identity to the amino acid sequence
of SEQ ID NO:55 (i.e., the amino acid sequence of the *I.
galbana* Δ4-desaturase shown in Figure 21). (All integers
between 30% and 100% similarity or identity are also included
within the scope of the present invention.)

20   The term "identity" refers to the relatedness of two
sequences on a nucleotide-by-nucleotide basis over a
particular comparison window or segment. Thus, identity is
defined as the degree of sameness, correspondence or
equivalence between the same strands (either sense or

25   antisense) of two DNA segments (or two amino acid sequences).
"Percentage of sequence identity" is calculated by comparing
two optimally aligned sequences over a particular region,
determining the number of positions at which the identical
base or amino acid occurs in both sequences in order to yield

30   the number of matched positions, dividing the number of such
positions by the total number of positions in the segment
being compared and multiplying the result by 100. Optimal
alignment of sequences may be conducted by the algorithm of
Smith & Waterman, Appl. Math. 2:482 (1981), by the algorithm

35   of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the

method of Pearson & Lipman, Proc. Natl. Acad. Sci. (USA)
85:2444 (1988) and by computer programs which implement the
relevant algorithms (e.g., Clustal Macaw Pileup
(http://cmgm.stanford.edu/biochem218/11Multiple.pdf; Higgins
5   et al., CABIOS. 5L151-153 (1989)), FASTDB (Intelligenetics),
BLAST (National Center for Biomedical Information; Altschul et
al., Nucleic Acids Research 25:3389-3402 (1997)), PILEUP
(Genetics Computer Group, Madison, WI) or GAP, BESTFIT, FASTA
and TFASTA (Wisconsin Genetics Software Package Release 7.0,
10 Genetics Computer Group, Madison, WI). (See U.S. Patent No.
5,912,120.)

      For purposes of the present invention, "complementarity
is defined as the degree of relatedness between two DNA
segments. It is determined by measuring the ability of the
15 sense strand of one DNA segment to hybridize with the
antisense strand of the other DNA segment, under appropriate
conditions, to form a double helix. A ''complement'' is
defined as a sequence which pairs to a given sequence based
upon the canonic base-pairing rules. For example, a sequence
20 A-G-T in one nucleotide strand is ''complementary'' to T-C-A in
the other strand.

      In the double helix, adenine appears in one strand,
thymine appears in the other strand. Similarly, wherever
guanine is found in one strand, cytosine is found in the
25 other. The greater the relatedness between the nucleotide
sequences of two DNA segments, the greater the ability to form
hybrid duplexes between the strands of the two DNA segments.

      "Similarity" between two amino acid sequences is defined
as the presence of a series of identical as well as conserved
30 amino acid residues in both sequences. The higher the degree
of similarity between two amino acid sequences, the higher the
correspondence, sameness or equivalence of the two sequences.
("Identity between two amino acid sequences is defined as the
presence of a series of exactly alike or invariant amino acid
35 residues in both sequences.) The definitions of

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"complementarity", "identity" and "similarity" are well known to those of ordinary skill in the art.

"Encoded by" refers to a nucleic acid sequence which codes for a polypeptide sequence, wherein the polypeptide sequence or a portion thereof contains an amino acid sequence of at least 3 amino acids, more preferably at least 8 amino acids, and even more preferably at least 15 amino acids from a polypeptide encoded by the nucleic acid sequence.

The present invention also encompasses an isolated nucleotide sequence which encodes PUFA desaturase activity and that is hybridizable, under moderately stringent conditions, to a nucleic acid having a nucleotide sequence comprising or complementary to the nucleotide sequences described above. A nucleic acid molecule is "hybridizable" to another nucleic acid molecule when a single-stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and ionic strength (see Sambrook et al., "Molecular Cloning: A Laboratory Manual, Second Edition (1989), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York)). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. "Hybridization" requires that two nucleic acids contain complementary sequences. However, depending on the stringency of the hybridization, mismatches between bases may occur. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation. Such variables are well known in the art. More specifically, the greater the degree of similarity or homology between two nucleotide sequences, the greater the value of Tm for hybrids of nucleic acids having those sequences. For hybrids of greater than 100 nucleotides in length, equations for calculating Tm have been derived (see Sambrook et al., *supra*). For hybridization with shorter nucleic acids, the position of mismatches becomes more

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important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., *supra*).

As used herein, an "isolated nucleic acid fragment or sequence" is a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. An isolated nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA. (A "fragment" of a specified polynucleotide refers to a polynucleotide sequence which comprises a contiguous sequence of approximately at least about 6 nucleotides, preferably at least about 8 nucleotides, more preferably at least about 10 nucleotides, and even more preferably at least about 15 nucleotides, and most preferable at least about 25 nucleotides identical or complementary to a region of the specified nucleotide sequence.) Nucleotides (usually found in their 5'-monophosphate form) are referred to by their single letter designation as follows: "A" for adenylate or deoxyadenylate (for RNA or DNA, respectively), "C" for cytidylate or deoxycytidylate, "G" for guanylate or deoxyguanylate, "U" for uridylate, "T" for deoxythymidylate, "R" for purines (A or G), "Y" for pyrimidines (C or T), "K" for G or T, "H" for A or C or T, "I" for inosine, and "N" for any nucleotide.

The terms "fragment or subfragment that is functionally equivalent" and "functionally equivalent fragment or subfragment" are used interchangeably herein. These terms refer to a portion or subsequence of an isolated nucleic acid fragment in which the ability to alter gene expression or produce a certain phenotype is retained whether or not the fragment or subfragment encodes an active enzyme. For example, the fragment or subfragment can be used in the design of chimeric constructs to produce the desired phenotype in a transformed plant. Chimeric constructs can be designed for use in co-suppression or antisense by linking a nucleic acid fragment or subfragment thereof, whether or not it encodes an

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active enzyme, in the appropriate orientation relative to a plant promoter sequence.

The terms "homology", "homologous", "substantially similar" and " corresponding substantially" are used
5   interchangeably herein. They refer to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate gene expression or produce a certain phenotype. These terms also refer to modifications of the nucleic acid fragments of
10   the instant invention such as deletion or insertion of one or more nucleotides that do not substantially alter the functional properties of the resulting nucleic acid fragment relative to the initial, unmodified fragment. It is therefore understood, as those skilled in the art will appreciate, that
15   the invention encompasses more than the specific exemplary sequences.

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding
20   sequences) the coding sequence.

"Native gene" refers to a gene as found in nature with its own regulatory sequences. In contrast,"chimeric construct" refers to a combination of nucleic acid fragments that are not normally found together in nature. Accordingly, a chimeric
25   construct may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that normally found in nature. (The term "isolated" means that the
30   sequence is removed from its natural environment.)

A "foreign" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric

constructs. A "transgene" is a gene that has been introduced
into the genome by a transformation procedure.

"Coding sequence" refers to a DNA sequence that codes for
a specific amino acid sequence. "Regulatory sequences" refer

5   to nucleotide sequences located upstream (5' non-coding
sequences), within, or downstream (3' non-coding sequences) of
a coding sequence, and which influence the transcription, RNA
processing or stability, or translation of the associated
coding sequence. Regulatory sequences may include, but are

10   not limited to, promoters, translation leader sequences,
introns, and polyadenylation recognition sequences.

"Promoter" refers to a DNA sequence capable of
controlling the expression of a coding sequence or functional
RNA. The promoter sequence consists of proximal and more

15   distal upstream elements, the latter elements often referred
to as enhancers. Accordingly, an "enhancer" is a DNA sequence
which can stimulate promoter activity and may be an innate
element of the promoter or a heterologous element inserted to
enhance the level or tissue-specificity of a promoter.

20   Promoter sequences can also be located within the transcribed
portions of genes, and/or downstream of the transcribed
sequences. Promoters may be derived in their entirety from a
native gene, or be composed of different elements derived from
different promoters found in nature, or even comprise

25   synthetic DNA segments. It is understood by those skilled in
the art that different promoters may direct the expression of
a gene in different tissues or cell types, or at different
stages of development, or in response to different
environmental conditions. Promoters which cause a gene to be

30   expressed in most host cell types at most times are commonly
referred to as "constitutive promoters". New promoters of
various types useful in plant cells are constantly being
discovered; numerous examples may be found in the compilation
by Okamuro and Goldberg, (1989) *Biochemistry of Plants*

35   *15*:1-82. It is further recognized that since in most cases

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the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of some variation may have identical promoter activity.

An "intron" is an intervening sequence in a gene that does not encode a portion of the protein sequence. Thus, such sequences are transcribed into RNA but are then excised and are not translated. The term is also used for the excised RNA sequences. An "exon" is a portion of the gene sequence that is transcribed and is found in the mature messenger RNA derived from the gene, but is not necessarily a part of the sequence that encodes the final gene product.

The "translation leader sequence" refers to a DNA sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner, R. and Foster, G. D. (1995) *Molecular Biotechnology* 3:225).

The "3' non-coding sequences" refer to DNA sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al., (1989) *Plant Cell* 1:671-680.

"RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from post-transcriptional processing of the primary transcript and is referred to as the

mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into protein by the cell. "cDNA" refers to a DNA that is complementary to and synthesized from a mRNA template using the enzyme reverse

5   transcriptase. The cDNA can be single-stranded or converted into the double-stranded form using the Klenow fragment of DNA polymerase I. "Sense" RNA refers to RNA transcript that includes the mRNA and can be translated into protein within a cell or *in vitro*. "Antisense RNA" refers to an RNA transcript

10 that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (U.S. Patent No. 5,107,065). The complementarity of an antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-coding

15 sequence, introns, or the coding sequence. "Functional RNA" refers to antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes. The terms "complement" and "reverse complement" are used interchangeably herein with respect to mRNA transcripts, and

20 are meant to define the antisense RNA of the message.

The term "endogenous RNA" refers to any RNA which is encoded by any nucleic acid sequence present in the genome of the host prior to transformation with the recombinant construct of the present invention, whether naturally-

25 occurring or non-naturally occurring, i.e., introduced by recombinant means, mutagenesis, etc.

The term "non-naturally occurring" means artificial, not consistent with what is normally found in nature.

The term "operably linked" refers to the association of

30 nucleic acid sequences on a single nucleic acid fragment so that the function of one is regulated by the other. For example, a promoter is operably linked with a coding sequence when it is capable of regulating the expression of that coding sequence (i.e., that the coding sequence is under the

35 transcriptional control of the promoter). Coding sequences

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can be operably linked to regulatory sequences in a sense or
antisense orientation. In another example, the complementary
RNA regions of the invention can be operably linked, either
directly or indirectly, 5' to the target mRNA, or 3' to the
5   target mRNA, or within the target mRNA, or a first
complementary region is 5' and its complement is 3' to the
target mRNA.

The term "expression", as used herein, refers to the
production of a functional end-product. Expression of a gene
10   involves transcription of the gene and translation of the mRNA
into a precursor or mature protein. "Antisense inhibition"
refers to the production of antisense RNA transcripts capable
of suppressing the expression of the target protein.
"Co-suppression" refers to the production of sense RNA
15   transcripts capable of suppressing the expression of identical
or substantially similar foreign or endogenous genes (U.S.
Patent No. 5,231,020).

"Mature" protein refers to a post-translationally
processed polypeptide; i.e., one from which any pre- or
20   propeptides present in the primary translation product have
been removed. "Precursor" protein refers to the primary
product of translation of mRNA; i.e., with pre- and pro-
peptides still present. Pre- and pro-peptides may be but are
not limited to intracellular localization signals.

25   "Stable transformation" refers to the transfer of a
nucleic acid fragment into a genome of a host organism,
resulting in genetically stable inheritance. In contrast,
"transient transformation" refers to the transfer of a nucleic
acid fragment into the nucleus, or DNA-containing organelle,
30   of a host organism resulting in gene expression without
integration or stable inheritance. Host organisms containing
the transformed nucleic acid fragments are referred to as
"transgenic" organisms. The preferred method of cell
transformation of rice, corn and other monocots is the use of
35   particle-accelerated or "gene gun" transformation technology

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(Klein et al., (1987) *Nature (London)* 327:70-73; U.S. Patent
No. 4,945,050), or an *Agrobacterium*-mediated method using an
appropriate Ti plasmid containing the transgene (Ishida Y.
et al., 1996, Nature Biotech. 14:745-750). The term

5   "transformation" as used herein refers to both stable
transformation and transient transformation.

Standard recombinant DNA and molecular cloning techniques
used herein are well known in the art and are described more
fully in Sambrook, J., Fritsch, E.F. and Maniatis, T.

10   *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor
Laboratory Press: Cold Spring Harbor, 1989 (hereinafter
"Sambrook").

The term "recombinant" refers to an artificial
combination of two otherwise separated segments of sequence,

15   e.g., by chemical synthesis or by the manipulation of isolated
segments of nucleic acids by genetic engineering techniques.

"PCR" or "Polymerase Chain Reaction" is a technique for
the synthesis of large quantities of specific DNA segments,
consists of a series of repetitive cycles (Perkin Elmer Cetus

20   Instruments, Norwalk, CT). Typically, the double stranded DNA
is heat denatured, the two primers complementary to the
3' boundaries of the target segment are annealed at low
temperature and then extended at an intermediate temperature.
One set of these three consecutive steps is referred to as a

25   cycle.

Polymerase chain reaction ("PCR") is a powerful technique
used to amplify DNA millions of fold, by repeated replication
of a template, in a short period of time. (Mullis et al, *Cold
Spring Harbor Symp. Quant. Biol.* 51:263-273 (1986); Erlich

30   et al, European Patent Application 50,424; European Patent
Application 84,796; European Patent Application 258,017,
European Patent Application 237,362; Mullis, European Patent
Application 201,184, Mullis et al U.S. Patent No. 4,683,202;
Erlich, U.S. Patent No. 4,582,788; and Saiki et al, U.S.

35   Patent No. 4,683,194). The process utilizes sets of specific

in vitro synthesized oligonucleotides to prime DNA synthesis.
The design of the primers is dependent upon the sequences of
DNA that are desired to be analyzed. The technique is carried
out through many cycles (usually 20-50) of melting the
5   template at high temperature, allowing the primers to anneal
to complementary sequences within the template and then
replicating the template with DNA polymerase.

The products of PCR reactions are analyzed by separation
in agarose gels followed by ethidium bromide staining and
10 visualization with UV transillumination. Alternatively,
radioactive dNTPs can be added to the PCR in order to
incorporate label into the products. In this case the
products of PCR are visualized by exposure of the gel to x-ray
film. The added advantage of radiolabeling PCR products is
15 that the levels of individual amplification products can be
quantitated.

The terms "recombinant construct", "expression construct"
and "recombinant expression construct" are used
interchangeably herein. These terms refer to a functional unit
20 of genetic material that can be inserted into the genome of a
cell using standard methodology well known to one skilled in
the art. Such construct may be itself or may be used in
conjunction with a vector. If a vector is used then the
choice of vector is dependent upon the method that will be
25 used to transform host plants as is well known to those
skilled in the art. For example, a plasmid vector can be
used. The skilled artisan is well aware of the genetic
elements that must be present on the vector in order to
successfully transform, select and propagate host cells
30 comprising any of the isolated nucleic acid fragments of the
invention. The skilled artisan will also recognize that
different independent transformation events will result in
different levels and patterns of expression (Jones et al.,
(1985) *EMBO J.* 4:2411-2418; De Almeida et al., (1989) *Mol.*
35 *Gen. Genetics 218*:78-86), and thus that multiple events must

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be screened in order to obtain lines displaying the desired
expression level and pattern. Such screening may be
accomplished by Southern analysis of DNA, Northern analysis of
mRNA expression, Western analysis of protein expression, or
5 phenotypic analysis.

Production of the Δ4-Desaturase Enyzme

        Once the gene encoding the Δ4-desaturase enzyme has been
isolated, it may then be introduced into either a prokaryotic
10 or eukaryotic host cell through the use of a vector or
construct. The vector, for example, a bacteriophage, cosmid
or plasmid, may comprise the nucleotide sequence encoding the
Δ4-desaturase enzyme, as well as any regulatory sequence
(e.g., promoter) which is functional in the host cell and is
15 able to elicit expression of the desaturase encoded by the
nucleotide sequence. The regulatory sequence (e.g., promoter)
is in operable association with or operably linked to the
nucleotide sequence. (A promoter is said to be "operably
linked" with a coding sequence if the promoter affects
20 transcription or expression of the coding sequence.) Suitable
promoters include, for example, those from genes encoding
alcohol dehydrogenase, glyceraldehyde-3-phosphate
dehydrogenase, phosphoglucoisomerase, phosphoglycerate kinase,
acid phosphatase, T7, TPI, lactase, metallothionein,
25 cytomegalovirus immediate early, whey acidic protein,
glucoamylase, and promoters activated in the presence of
galactose, for example, GAL1 and GAL10. Additionally,
nucleotide sequences which encode other proteins,
oligosaccharides, lipids, etc. may also be included within the
30 vector as well as other regulatory sequences such as a
polyadenylation signal (e.g., the poly-A signal of SV-40T-
antigen, ovalalbumin or bovine growth hormone). The choice of
sequences present in the construct is dependent upon the
desired

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expression products as well as the nature of the host cell.

   As noted above, once the vector has been constructed, it
may then be introduced into the host cell of choice by methods
known to those of ordinary skill in the art including, for
example, transfection, transformation and electroporation (see
Molecular Cloning: A Laboratory Manual, 2nd ed., Vol. 1-3, ed.
Sambrook et al., Cold Spring Harbor Laboratory Press (1989)).
The host cell is then cultured under suitable conditions
permitting expression of the genes leading to the production
of the desired PUFA, which is then recovered and purified.

   Examples of suitable prokaryotic host cells include, for
example, bacteria such as Escherichia coli, Bacillus subtilis
as well as Cyanobacteria such as Spirulina spp. (i.e., blue-
green algae). Examples of suitable eukaryotic host cells
include, for example, mammalian cells, plant cells, yeast
cells such as Saccharomyces cerevisiae, Saccharomyces
carlsbergensis, Lipomyces starkey, Candida spp. such as
Yarrowia (Candida) lipolytica, Kluyveromyces spp., Pichia
spp., Trichoderma spp. or Hansenula spp., or fungal cells such
as filamentous fungal cells, for example, Aspergillus,
Neurospora and Penicillium. Preferably, Saccharomyces
cerevisiae (baker's yeast) cells are utilized.

   Expression in a host cell can be accomplished in a
transient or stable fashion. Transient expression can occur
from introduced constructs which contain expression signals
functional in the host cell, but which constructs do not
replicate and rarely integrate in the host cell, or when the
host cell is not proliferating. Transient expression also can
be accomplished by inducing the activity of a regulatable
promoter operably linked to the gene of interest, although
such inducible systems frequently exhibit a low basal level of
expression. Stable expression can be achieved by introduction
of a construct that can integrate into the host genome or that
autonomously replicates in the host cell. Stable expression
of the gene of interest can be selected through the use of a

selectable marker located on or transfected with the
expression construct, followed by selection for cells
expressing the marker. When stable expression results from
integration, the site of the construct's integration can occur
5   randomly within the host genome or can be targeted through the
use of constructs containing regions of homology with the host
genome sufficient to target recombination with the host locus.
Where constructs are targeted to an endogenous locus, all or
some of the transcriptional and translational regulatory
10   regions can be provided by the endogenous locus.

        A transgenic mammal may also be used in order to express
the enzyme of interest (i.e., Δ4-desaturase), and ultimately
the PUFA(s) of interest. More specifically, once the above-
described construct is created, it may be inserted into the
15   pronucleus of an embryo. The embryo may then be implanted
into a recipient female. Alternatively, a nuclear transfer
method could also be utilized (Schnieke et al., Science
278:2130-2133 (1997)). Gestation and birth are then permitted
(see, e.g., U.S. Patent No. 5,750,176 and U.S. Patent No.
20   5,700,671). Milk, tissue or other fluid samples from the
offspring should then contain altered levels of PUFAs, as
compared to the levels normally found in the non-transgenic
animal. Subsequent generations may be monitored for
production of the altered or enhanced levels of PUFAs and thus
25   incorporation of the gene encoding the desired desaturase
enzyme into their genomes. The mammal utilized as the host
may be selected from the group consisting of, for example, a
mouse, a rat, a rabbit, a pig, a goat, a sheep, a horse and a
cow. However, any mammal may be used provided it has the
30   ability to incorporate DNA encoding the enzyme of interest
into its genome.

        For expression of a desaturase polypeptide, functional
transcriptional and translational initiation and termination
regions are operably linked to the DNA encoding the desaturase
35   polypeptide. Transcriptional and translational initiation and

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termination regions are derived from a variety of nonexclusive
sources, including the DNA to be expressed, genes known or
suspected to be capable of expression in the desired system,
expression vectors, chemical synthesis, or from an endogenous
5   locus in a host cell. Expression in a plant tissue and/or
plant part presents certain efficiencies, particularly where
the tissue or part is one which is harvested early, such as
seed, leaves, fruits, flowers, roots, etc. Expression can be
targeted to that location with the plant by utilizing specific
10   regulatory sequence such as those of U.S. Patent Nos.
5,463,174, 4,943,674, 5,106,739, 5,175,095, 5,420,034,
5,188,958, and 5,589,379. Alternatively, the expressed
protein can be an enzyme which produces a product which may be
incorporated, either directly or upon further modifications,
15   into a fluid fraction from the host plant. Expression of a
desaturase gene, or antisense desaturase transcripts, can
alter the levels of specific PUFAs, or derivatives thereof,
found in plant parts and/or plant tissues. The desaturase
polypeptide coding region may be expressed either by itself or
20   with other genes, in order to produce tissues and/or plant
parts containing higher proportions of desired PUFAs or in
which the PUFA composition more closely resembles that of
human breast milk (Prieto et al., PCT publication WO
95/24494). The termination region may be derived from the 3'
25   region of the gene from which the initiation region was
obtained or from a different gene. A large number of
termination regions are known to and have been found to be
satisfactory in a variety of hosts from the same and different
genera and species. The termination region usually is
30   selected as a matter of convenience rather than because of any
particular property.

        As noted above, a plant (e.g., Glycine max (soybean) or
Brassica napus (canola)) or plant tissue may also be utilized
as a host or host cell, respectively, for expression of the
35   desaturase enzyme which may, in turn, be utilized in the

production of polyunsaturated fatty acids. More specifically, desired PUFAS can be expressed in seed. Methods of isolating seed oils are known in the art. Thus, in addition to providing a source for PUFAs, seed oil components may be

5   manipulated through the expression of the desaturase gene, as well as perhaps other desaturase genes and elongase genes, in order to provide seed oils that can be added to nutritional compositions, pharmaceutical compositions, animal feeds and cosmetics. Once again, a vector which comprises a DNA

10   sequence encoding the desaturase operably linked to a promoter, will be introduced into the plant tissue or plant for a time and under conditions sufficient for expression of the desaturase gene. The vector may also comprise one or more genes that encode other enzymes, for example, Δ5-desaturase,

15   elongase, Δ12-desaturase, Δ15-desaturase, Δ17-desaturase, and/or Δ19-desaturase. The plant tissue or plant may produce the relevant substrate (e.g., adrenic acid or ω3-docosapentaenoic acid) upon which the enzyme acts or a vector encoding enzymes which produce such substrates may be

20   introduced into the plant tissue, plant cell or plant. In addition, substrate may be sprayed on plant tissues expressing the appropriate enzymes. Using these various techniques, one may produce PUFAs (e.g., n-6 unsaturated fatty acids such as ω6-docosapentaenoic acid, or n-3 fatty acids such as

25   docosahexaenoic acid) by use of a plant cell, plant tissue or plant. It should also be noted that the invention also encompasses a transgenic plant comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid

30   in, for example, the seeds of the transgenic plant.

        The regeneration, development, and cultivation of plants from single plant protoplast transformants or from various transformed explants is well known in the art (Weissbach and Weissbach, In: Methods for Plant Molecular

35   Biology, (Eds.), Academic Press, Inc. San Diego, CA, (1988)).

This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of embryonic development through the rooted plantlet stage. Transgenic
5   embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

The development or regeneration of plants containing the foreign, exogenous gene that encodes a protein of interest is
10   well known in the art. Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants. Otherwise, pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important lines. Conversely, pollen from plants of these important lines is
15   used to pollinate regenerated plants. A transgenic plant of the present invention containing a desired polypeptide is cultivated using methods well known to one skilled in the art.

There are a variety of methods for the regeneration of plants from plant tissue. The particular method of
20   regeneration will depend on the starting plant tissue and the particular plant species to be regenerated.

Methods for transforming dicots, primarily by use of *Agrobacterium tumefaciens*, and obtaining transgenic plants have been published for cotton (U.S. Patent No. 5,004,863,
25   U.S. Patent No. 5,159,135, U.S. Patent No. 5,518,908); soybean (U.S. Patent No. 5,569,834, U.S. Patent No. 5,416,011, McCabe et. al., BiolTechnology 6:923 (1988), Christou et al., Plant Physiol. 87:671-674 (1988)); Brassica (U.S. Patent No. 5,463,174); peanut (Cheng et al., Plant Cell Rep.
30   15:653-657 (1996), McKently et al., Plant Cell Rep. 14:699-703 (1995)); papaya; and pea (Grant et al., Plant Cell Rep. 15:254-258, (1995)).

Transformation of monocotyledons using electroporation, particle bombardment, and Agrobacterium have also been
35   reported. Transformation and plant regeneration have been

achieved in asparagus (Bytebier et al., Proc. Natl. Acad. Sci. (USA) 84:5354, (1987)); barley (Wan and Lemaux, Plant Physiol 104:37 (1994)); Zea mays (Rhodes et al., Science 240:204 (1988), Gordon-Kamm et al., Plant Cell 2:603-618 (1990), Fromm et al., BiolTechnology 8:833 (1990), Koziel et al., BiolTechnology 11:194, (1993), Armstrong et al., Crop Science 35:550-557 (1995)); oat (Somers et al., BiolTechnology 10:1589 (1992)); orchard grass (Horn et al., Plant Cell Rep. 7:469 (1988)); rice (Toriyama et al., TheorAppl. Genet. 205:34 (1986); Part et al., Plant Mol. Biol. 32:1135-1148, (1996); Abedinia et al., Aust. J. Plant Physiol. 24:133-141 (1997); Zhang and Wu, Theor. Appl. Genet. 76:835 (1988); Zhang et al. Plant Cell Rep. 7:379, (1988); Battraw and Hall, Plant Sci. 86:191-202 (1992); Christou et al., Bio/Technology 9:957 (1991)); rye (De la Pena et al., Nature 325:274 (1987)); sugarcane (Bower and Birch, Plant J. 2:409 (1992)); tall fescue (Wang et al., BiolTechnology 10:691 (1992)), and wheat (Vasil et al., BiolTechnology 10:667 (1992); U.S. Patent No. 5,631,152).

Assays for gene expression based on the transient expression of cloned nucleic acid constructs have been developed by introducing the nucleic acid molecules into plant cells by polyethylene glycol treatment, electroporation, or particle bombardment (Marcotte et al., Nature 335:454-457 (1988); Marcotte et al., Plant Cell 1:523-532 (1989); McCarty et al., Cell 66:895-905 (1991); Hattori et al., Genes Dev. 6:609-618 (1992); Goff et al., EMBO J. 9:2517-2522 (1990)).

Transient expression systems may be used to functionally dissect gene constructs (see generally, Maliga et al., Methods in Plant Molecular Biology, Cold Spring Harbor Press (1995)). It is understood that any of the nucleic acid molecules of the present invention can be introduced into a plant cell in a permanent or transient manner in combination with other genetic elements such as vectors, promoters, enhancers etc.

In addition to the above discussed procedures, practitioners are familiar with the standard resource materials which describe specific conditions and procedures for the construction, manipulation and isolation of

5   macromolecules (e.g., DNA molecules, plasmids, etc.), generation of recombinant organisms and the screening and isolating of clones, (see for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989); Maliga et al., Methods in Plant Molecular

10   Biology, Cold Spring Harbor Press (1995); Birren et al., Genome Analysis: Detecting Genes, 1, Cold Spring Harbor, New York (1998); Birren et al., Genome Analysis: Analyzing DNA, 2, Cold Spring Harbor, New York (1998); Plant Molecular Biology: A Laboratory Manual, eds. Clark, Springer, New York (1997)).

15   The substrates which may be produced by the host cell either naturally or transgenically, as well as the enzymes which may be encoded by DNA sequences present in the vector which is subsequently introduced into the host cell, are shown in Figure 1.

20

Uses of the Δ4-Desaturase Gene and Enzyme Encoded Thereby

         As noted above, the isolated desaturase genes and the desaturase enzymes encoded thereby have many uses. For example, the gene and corresponding enzyme may be used

25   indirectly or directly in the production of polyunsaturated fatty acids, for example, Δ4-desaturase may be used in the production of ω6-docosapentaenoic acid or docosahexaenoic acid. "Directly" is meant to encompass the situation where the enzyme directly converts the acid to another acid, the latter

30   of which is utilized in a composition (e.g., the conversion of adrenic acid to ω6-docosapentaenoic acid). "Indirectly" is meant to encompass the situation where an acid is converted to another acid (i.e., a pathway intermediate) by the desaturase (e.g., adrenic acid to ω6-docosapentaenoic acid) and then the

35   latter acid is converted to another acid by use of a

desaturase or non-desaturase enzyme (e.g., ω6-docosapentaenoic acid to docosahexaenoic acid by Δ19-desaturase). Also, the present invention includes "indirect" situations in which the PUFA is first converted to another polyunsaturated fatty acid

5   by a non-Δ4-desaturase enzyme (for example, an elongase or another desaturase) and then converted to a final product via Δ4-desaturase. For example, eicosapentaenoic acid may be converted to ω3-docosapentaenoic acid by an elongase, and then converted to docosahexaenoic acid by a Δ4-desaturase. These

10 polyunsaturated fatty acids (i.e., those produced either directly or indirectly by activity of the desaturase enzyme) may be added to, for example, nutritional compositions, pharmaceutical compositions, cosmetics, and animal feeds, all of which are encompassed by the present invention. These uses

15 are described, in detail, below.

Nutritional Compositions

    The present invention includes nutritional compositions. Such compositions, for purposes of the present invention,

20 include any food or preparation for human consumption including for enteral or parenteral consumption, which when taken into the body (a) serve to nourish or build up tissues or supply energy and/or (b) maintain, restore or support adequate nutritional status or metabolic function.

25   The nutritional composition of the present invention comprises at least one oil or acid produced directly or indirectly by use of the desaturase gene, in accordance with the present invention, and may either be in a solid or liquid form. Additionally, the composition may include edible

30 macronutrients, vitamins and minerals in amounts desired for a particular use. The amount of such ingredients will vary depending on whether the composition is intended for use with normal, healthy infants, children or adults having specialized needs such as those which accompany certain metabolic

35 conditions (e.g., metabolic disorders).

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Examples of macronutrients which may be added to the
composition include but are not limited to edible fats,
carbohydrates and proteins. Examples of such edible fats
include but are not limited to coconut oil, borage oil, fungal
5   oil, black current oil, soy oil, and mono- and diglycerides.
Examples of such carbohydrates include but are not limited to
glucose, edible lactose and hydrolyzed search. Additionally,
examples of proteins which may be utilized in the nutritional
composition of the invention include but are not limited to
10   soy proteins, electrodialysed whey, electrodialysed skim milk,
milk whey, or the hydrolysates of these proteins.

With respect to vitamins and minerals, the following may
be added to the nutritional compositions of the present
invention: calcium, phosphorus, potassium, sodium, chloride,
15   magnesium, manganese, iron, copper, zinc, selenium, iodine,
and Vitamins A, E, D, C, and the B complex. Other such
vitamins and minerals may also be added.

The components utilized in the nutritional compositions
of the present invention will be of semi-purified or purified
20   origin. By semi-purified or purified is meant a material
which has been prepared by purification of a natural material
or by synthesis.

Examples of nutritional compositions of the present
invention include but are not limited to infant formulas,
25   dietary supplements (e.g., adult nutritional products and
oil), dietary substitutes, and rehydration compositions.
Nutritional compositions of particular interest include but
are not limited to those utilized for enteral and parenteral
supplementation for infants, specialist infant formulas,
30   supplements for the elderly, and supplements for those with
gastrointestinal difficulties and/or malabsorption.

The nutritional composition of the present invention may
also be added to food even when supplementation of the diet is
not required. For example, the composition may be added to
35   food of any type including but not limited to margarines,

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modified butters, cheeses, milk, yogurt, chocolate, candy, snacks, salad oils, cooking oils, cooking fats, meats, fish and beverages.

5   In a preferred embodiment of the present invention, the nutritional composition is an enteral nutritional product, more preferably, an adult or pediatric enteral nutritional product. This composition may be administered to adults or children experiencing stress or having specialized needs due to chronic or acute disease states. The composition may

10 comprise, in addition to polyunsaturated fatty acids produced in accordance with the present invention, macronutrients, vitamins and minerals as described above. The macronutrients may be present in amounts equivalent to those present in human milk or on an energy basis, i.e., on a per calorie basis.

15   Methods for formulating liquid or solid enteral and parenteral nutritional formulas are well known in the art. (See also the Examples below.)

The enteral formula, for example, may be sterilized and subsequently utilized on a ready-to-feed (RTF) basis or stored

20 in a concentrated liquid or powder. The powder can be prepared by spray drying the formula prepared as indicated above, and reconstituting it by rehydrating the concentrate. Adult and pediatric nutritional formulas are well known in the art and are commercially available (e.g., Similac®, Ensure®,

25 Jevity® and Alimentum® from Ross Products Division, Abbott Laboratories, Columbus, Ohio). An oil or acid produced in accordance with the present invention may be added to any of these formulas.

The energy density of the nutritional compositions of the

30 present invention, when in liquid form, may range from about 0.6 Kcal to about 3 Kcal per ml. When in solid or powdered form, the nutritional supplements may contain from about 1.2 to more than 9 Kcals per gram, preferably about 3 to 7 Kcals per gm. In general, the osmolality of a liquid product should

be less than 700 mOsm and, more preferably, less than 660
mOsm.

The nutritional formula may include macronutrients,
vitamins, and minerals, as noted above, in addition to the
5   PUFAs produced in accordance with the present invention. The
presence of these additional components helps the individual
ingest the minimum daily requirements of these elements. In
addition to the provision of PUFAs, it may also be desirable
to add zinc, copper, folic acid and antioxidants to the
10 composition. It is believed that these substances boost a
stressed immune system and will therefore provide further
benefits to the individual receiving the composition. A
pharmaceutical composition may also be supplemented with these
elements.

15   In a more preferred embodiment, the nutritional
composition comprises, in addition to antioxidants and at
least one PUFA, a source of carbohydrate wherein at least 5
weight percent of the carbohydrate is indigestible
oligosaccharide. In a more preferred embodiment, the
20 nutritional composition additionally comprises protein,
taurine, and carnitine.

As noted above, the PUFAs produced in accordance with the
present invention, or derivatives thereof, may be added to a
dietary substitute or supplement, particularly an infant
25 formula, for patients undergoing intravenous feeding or for
preventing or treating malnutrition or other conditions or
disease states. As background, it should be noted that human
breast milk has a fatty acid profile comprising from about
0.15% to about 0.36% as DHA, from about 0.03% to about 0.13%
30 as EPA, from about 0.30% to about 0.88% as AA, from about
0.22% to about 0.67% as DGLA, and from about 0.27% to about
1.04% as GLA. Thus, fatty acids such as AA, EPA and/or
docosahexaenoic acid (DHA), produced in accordance with the
present invention, can be used to alter, for example, the
35 composition of infant formulas in order to better replicate

the PUFA content of human breast milk or to alter the presence
of PUFAs normally found in a non-human mammal's milk. In
particular, a composition for use in a pharmacologic or food
supplement, particularly a breast milk substitute or
supplement, will preferably comprise one or more of AA, DGLA
and GLA. More preferably, the oil will comprise from about
0.3 to 30% AA, from about 0.2 to 30% DGLA, and/or from about
0.2 to about 30% GLA.

Parenteral nutritional compositions comprising from about
2 to about 30 weight percent fatty acids calculated as
triglycerides are encompassed by the present invention. The
preferred composition has about 1 to about 25 weight percent
of the total PUFA composition as GLA (U.S. Patent No.
5,196,198). Other vitamins, particularly fat-soluble vitamins
such as vitamin A, D, E and L-carnitine can optionally be
included. When desired, a preservative such as alpha-
tocopherol may be added in an amount of about 0.1% by weight.

In addition, the ratios of AA, DGLA and GLA can be
adapted for a particular given end use. When formulated as a
breast milk supplement or substitute, a composition which
comprises one or more of AA, DGLA and GLA will be provided in
a ratio of about 1:19:30 to about 6:1:0.2, respectively. For
example, the breast milk of animals can vary in ratios of
AA:DGLA:GLA ranging from 1:19:30 to 6:1:0.2, which includes
intermediate ratios which are preferably about 1:1:1, 1:2:1,
1:1:4. When produced together in a host cell, adjusting the
rate and percent of conversion of a precursor substrate such
as GLA and DGLA to AA can be used to precisely control the
PUFA ratios. For example, a 5% to 10% conversion rate of DGLA
to AA can be used to produce an AA to DGLA ratio of about
1:19, whereas a conversion rate of about 75% TO 80% can be
used to produce an AA to DGLA ratio of about 6:1. Therefore,
whether in a cell culture system or in a host animal,
regulating the timing, extent and specificity of desaturase
expression, as well as the expression of other desaturases and

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elongases, can be used to modulate PUFA levels and ratios. The PUFAs produced in accordance with the present invention (e.g., AA and EPA) may then be combined with other PUFAs/acids (e.g., GLA) in the desired concentrations and ratios.

Additionally, PUFA produced in accordance with the present invention or host cells containing them may also be used as animal food supplements to alter an animal's tissue or milk fatty acid composition to one more desirable for human or animal consumption.

Pharmaceutical Compositions

The present invention also encompasses a pharmaceutical composition comprising one or more of the acids and/or resulting oils produced using the desaturase genes, in accordance with the methods described herein. More specifically, such a pharmaceutical composition may comprise one or more of the acids and/or oils as well as a standard, well-known, non-toxic pharmaceutically acceptable carrier, adjuvant or vehicle such as, for example, phosphate buffered saline, water, ethanol, polyols, vegetable oils, a wetting agent or an emulsion such as a water/oil emulsion. The composition may be in either a liquid or solid form. For example, the composition may be in the form of a tablet, capsule, ingestible liquid or powder, injectible, or topical ointment or cream. Proper fluidity can be maintained, for example, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. It may also be desirable to include isotonic agents, for example, sugars, sodium chloride and the like. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening agents, flavoring agents and perfuming agents.

Suspensions, in addition to the active compounds, may comprise suspending agents such as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan

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esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth or mixtures of these substances.

Solid dosage forms such as tablets and capsules can be
5   prepared using techniques well known in the art. For example, PUFAs produced in accordance with the present invention can be tableted with conventional tablet bases such as lactose, sucrose, and cornstarch in combination with binders such as acacia, cornstarch or gelatin, disintegrating agents such as
10 potato starch or alginic acid, and a lubricant such as stearic acid or magnesium stearate. Capsules can be prepared by incorporating these excipients into a gelatin capsule along with antioxidants and the relevant PUFA(s). The antioxidant and PUFA components should fit within the guidelines presented
15 above.

For intravenous administration, the PUFAs produced in accordance with the present invention or derivatives thereof may be incorporated into commercial formulations such as Intralipids™. The typical normal adult plasma fatty acid
20 profile comprises 6.64 to 9.46% of AA, 1.45 to 3.11% of DGLA, and 0.02 to 0.08% of GLA. These PUFAs or their metabolic precursors can be administered alone or in combination with other PUFAs in order to achieve a normal fatty acid profile in a patient. Where desired, the individual components of the
25 formulations may be provided individually, in kit form, for single or multiple use. A typical dosage of a particular fatty acid is from 0.1 mg to 20 g (up to 100 g) daily and is preferably from 10 mg to 1, 2, 5 or 10 g daily.

Possible routes of administration of the pharmaceutical
30 compositions of the present invention include, for example, enteral (e.g., oral and rectal) and parenteral. For example, a liquid preparation may be administered, for example, orally or rectally. Additionally, a homogenous mixture can be completely dispersed in water, admixed under sterile
35 conditions with physiologically acceptable diluents,

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preservatives, buffers or propellants in order to form a spray or inhalant.

The route of administration will, of course, depend upon the desired effect. For example, if the composition is being

5   utilized to treat rough, dry, or aging skin, to treat injured or burned skin, or to treat skin or hair affected by a disease or condition, it may perhaps be applied topically.

The dosage of the composition to be administered to the patient may be determined by one of ordinary skill in the art

10 and depends upon various factors such as weight of the patient, age of the patient, immune status of the patient, etc.

With respect to form, the composition may be, for example, a solution, a dispersion, a suspension, an emulsion

15 or a sterile powder which is then reconstituted.

The present invention also includes the treatment of various disorders by use of the pharmaceutical and/or nutritional compositions described herein. In particular, the compositions of the present invention may be used to treat

20 restenosis after angioplasty. Furthermore, symptoms of inflammation, rheumatoid arthritis, asthma and psoriasis may also be treated with the compositions of the invention. Evidence also indicates that PUFAs may be involved in calcium metabolism; thus, the compositions of the present invention

25 may, perhaps, be utilized in the treatment or prevention of osteoporosis and of kidney or urinary tract stones.

Additionally, the compositions of the present invention may also be used in the treatment of cancer. Malignant cells have been shown to have altered fatty acid compositions.

30 Addition of fatty acids has been shown to slow their growth, cause cell death and increase their susceptibility to chemotherapeutic agents. Moreover, the compositions of the present invention may also be useful for treating cachexia associated with cancer.

The compositions of the present invention may also be used to treat diabetes (see U.S. Patent No. 4,826,877 and Horrobin et al., Am. J. Clin. Nutr. Vol. 57 (Suppl.) 732S-737S). Altered fatty acid metabolism and composition have been demonstrated in diabetic animals.

Furthermore, the compositions of the present invention, comprising PUFAs produced either directly or indirectly through the use of the desaturase enzymes, may also be used in the treatment of eczema, in the reduction of blood pressure, and in the improvement of mathematics examination scores. Additionally, the compositions of the present invention may be used in inhibition of platelet aggregation, induction of vasodilation, reduction in cholesterol levels, inhibition of proliferation of vessel wall smooth muscle and fibrous tissue (Brenner et al., Adv. Exp. Med. Biol. Vol. 83, p.85-101, 1976), reduction or prevention of gastrointestinal bleeding and other side effects of non-steroidal anti-inflammatory drugs (see U.S. Patent No. 4,666,701), prevention or treatment of endometriosis and premenstrual syndrome (see U.S. Patent No. 4,758,592), and treatment of myalgic encephalomyelitis and chronic fatigue after viral infections (see U.S. Patent No. 5,116,871).

Further uses of the compositions of the present invention include use in the treatment of AIDS, multiple sclerosis, and inflammatory skin disorders, as well as for maintenance of general health.

Additionally, the composition of the present invention may be utilized for cosmetic purposes. It may be added to pre-existing cosmetic compositions such that a mixture is formed or may be used as a sole composition.

Veterinary Applications

It should be noted that the above-described pharmaceutical and nutritional compositions may be utilized in connection with animals (i.e., domestic or non-domestic), as

well as humans, as animals experience many of the same needs
and conditions as humans. For example, the oil or acids of
the present invention may be utilized in animal feed
supplements, animal feed substitutes, animal vitamins or in
5   animal topical ointments.

        The present invention may be illustrated by the use of
the following non-limiting examples:

                            Example I
10   Design of Degenerate Oligonucleotides for the Isolation of
      Desaturases from _Thraustochytrium aureum_ and cDNA Library
                          Construction

        The fatty acid composition analysis of the marine fungus
15   _Thraustochytrium aureum_ (_T. aureum_) (ATCC 34304) was
investigated to determine the types and amounts of
polyunsaturated fatty acids (PUFAs). This fungus had
substantial amounts of longer chain PUFAs such as arachidonic
acid (ARA, 20:4n-6) and eicosapentaenoic acid (EPA, 20:5 n-3).
20   However, _T. aureum_ also produced PUFAs such as adrenic acid
(ADA, 22:4n-6), ω6-docosapentaenoic acid (ω6-DPA, 22:5n-6),
ω3-docosapentaenoic acid (ω3-DPA, 22:5n-3), with the highest
amount of fatty acid being docosahexaenoic acid (DHA, 22:6n-3)
(see Figure 1). Thus in addition to Δ6-, Δ5- and Δ17-
25   desaturases, _T. aureum_ probably contains a Δ19-desaturase
which converts ADA to ω3-DPA or ω6-DPA to DHA and/or a Δ4-
desaturase which desaturates ADA to ω6-DPA or ω3-DPA to DHA.
The goal was therefore to attempt to isolate the predicted
desaturase genes from _T. aureum_, and to verify the
30   functionality of the enzymes by expression in an alternate
host.

        To isolate genes encoding for functional desaturase
enzymes, a cDNA library was constructed. _T. aureum_ (ATCC
34304) cells were grown in BY+ Media (#790, Difco, Detroit,
35   MI) at room temperature for 4 days, in the presence of light,
and with constant agitation (250 rpm) to obtain the maximum
biomass. These cells were harvested by centrifugation at 5000
rpm for 10 minutes and rinsed in ice-cold RNase-free water.

These cells were then lysed in a French press at 10,000 psi, and the lysed cells were directly collected into TE buffered phenol. Proteins from the cell lysate were removed by repeated phenol: chloroform (1:1 v/v) extraction, followed by
5   a chloroform extraction. The nucleic acids from the aqueous phase were precipitated at -70°C for 30 minutes using 0.3M (final concentration) sodium acetate (pH 5.6) and one volume of isopropanol. The precipitated nucleic acids were collected by centrifugation at 15,000 rpm for 30 minutes at 4°C, vacuum-
10   dried for 5 minutes and then treated with DNaseI (RNase-free) in 1X DNase buffer (20 mM Tris-Cl, pH 8.0; 5mM MgCl$_2$) for 15 minutes at room temperature. The reaction was quenched with 5 mM EDTA (pH 8.0) and the RNA further purified using the Qiagen RNeasy Maxi kit (Qiagen, Valencia, CA) as per the
15   manufacturer's protocol.

    Messenger RNA was isolated from total RNA using oligo dT cellulose resin, and the pBluescript II XR library construction kit (Stratagene, La Jolla, CA) was used to synthesize double stranded cDNA which was then directionally
20   cloned (5' EcoRI/3' XhoI) into pBluescript II SK(+) vector (Stratagene, La Jolla, CA). The T. aureum library contained approximately 2.5 x 10^6 clones each with an average insert size of approximately 700 bp. Genomic DNA from PUFA-producing T. aureum cultures was isolated by crushing the culture in liquid
25   nitrogen and was purified using Qiagen Genomic DNA Extraction Kit (Qiagen, Valencia, CA).

    The approach taken was to design degenerate oligonucleotides (primers) that represent amino acid motifs that are conserved in known desaturases. These primers could
30   be then used in a PCR reaction to identify a fragment containing the conserved regions in the predicted desaturase genes from fungi. Since the only fungal desaturases which have been identified are Δ5- and Δ6-desaturase genes from Mortierella alpina (Genbank accession numbers AF067650,
35   AB020032, respectively), desaturase sequences from plants as well as animals were taken into consideration during the design of these degenerate primers. In particular, known Δ5- and Δ6-desaturase sequences from the following organisms were

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used for the design of these degenerate primers: *Mortierella alpina, Borago officinalis, Helianthus annuus, Brassica napus, Dictyostelium discoideum, Rattus norvegicus, Mus musculus, Homo sapiens, Caenorhabditis elegans, Arabidopsis thaliana,*

5   and *Ricinus communis.* The degenerate primers used were as follows using the CODEHOP Blockmaker program (http://blocks.fhcrc.org/codehop.html):

a. Protein motif 1: NH_3- VYDVTEWVKRHPGG -COOH (SEQ ID NO:56)
Primer RO 834 (SEQ ID NO:1):

10   5'-GTBTAYGAYGTBACCGARTGGGTBAAGCGYCAYCCBGGHGGH-3'
B. Protein Motif 2: NH_3- GASANWWKHQHNVHH -COOH (SEQ ID NO:57)
Primer RO835 (Forward)(SEQ ID NO:2):
5-'GGHGCYTCCGCYAACTGGTGGAAGCAYCAGCAYAACGTBCAYCAY-3'
Primer RO836 (Reverse)(SEQ ID NO:3):

15   5-'RTGRTGVACGTTRTGCTGRTGCTTCCACCAGTTRGCGGARGCDCC-3'
C. Protein Motif 3: NH_3- NYQIEHHLFPTM -COOH (SEQ ID NO:58)
Primer RO838 (Reverse) (SEQ ID NO:4)
5'-TTGATRGTCTARCTYGTRGTRGASAARGGVTGGTAC-3'

20   In addition, two more primers were designed based on the 2nd and 3rd conserved 'Histidine-box' found in known Δ6-desaturases. These were:
Primer RO753 (SEQ ID NO:5) 5'-CATCATCATXGGRAAXARRTGRTG-3'
Primer RO754 (SEQ ID NO:6) 5'-CTACTACTACTACAYCAYACXTAY ACXAAY-

25   3'.
The degeneracy code for the oligonucleotide sequences was:
B=C,G,T; H=A,C,T; S=C,G; R=A,G; V=A,C,G; Y=C,T; D= A,T,C; X= A,C,G,T

30   Example II
                    Use of Degenerate Oligonucleotides
                 for the Isolation of a Desaturase from a Fungus

       To isolate putative desaturase genes, total RNA was
35   isolated using the lithium chloride method (Hoge, *et al.* (1982) Exp. Mycol. 6:225-232). Approximately 5 μg was reverse transcribed using the SuperScript Preamplification system (LifeTechnologies, Rockville, MD) to produce first strand cDNA. The following primer combinations were used: RO834/836,

RO834/838, RO835/836, RO835/838 and RO753/754 were used in several PCR reactions with different thermocycling parameters and Taq polymerase at annealing temperatures below 60°C, but no bands were produced.

5   In additional attempts to isolate fragments of desaturases, the degenerate primers RO834 /838 (designed with the block maker program) and RO753/754 were used in a 50 µl reaction. The following components were combined: 2 µl of the first strand cDNA template, 20mM Tris-HCl, pH 8.4, 50mM KCl,

10   1.5mM $MgCl_2$, 200 µM each deoxyribonucleotide triphosphate, 0.2 pmole final concentration of each primer and cDNA polymerase (Clonetech, Palo Alto, CA). Thermocycling was carried out as follows: an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of; denaturation at 94°C for 30 seconds,

15   annealing at 60°C for 30 seconds and extension at 72°C for 1 minute. This was followed by a final extension at 72°C for 7 minutes. Two faint bands of approximately 1000 bp were detected for primers RO834 /838, while a slightly smaller but more intense band of 800-900 bp was found with the primer pair

20   RO753/754. The reactions were separated on a 1% agarose gel, excised, and purified with the QiaQuick Gel Extraction Kit (Qiagen, Valencia, CA). The staggered ends on these fragments were 'filled-in' using T4 DNA polymerase (LifeTechnologies, Rockville, MD) as per manufacture's specifications, and these

25   DNA fragments were cloned into the PCR-Blunt vector (Invitrogen, Carlsbad, CA). The recombinant plasmids were transformed into TOP10 supercompetent cells (Invitrogen, Carlsbad, CA) and clones were partially sequenced.

     Subsequently, the sequences of clone 30-3 (reaction with

30   RO834 /838) and clone 17-1(reaction with RO753/754) were found to overlap to create a 1313 bp fragment. The fragment was translated and Tfasta used to search the GenBank database. The highest match was *Mortierella alpina* Δ5-desaturase (Genbank accession # AF067654) (27% homology in 202 amino acids),

35   *Spirulina platensis* Δ6-desaturase (Genbank accession number X87094) (30% homology in 121 amino acids), *Dictyostelium discoideum* Δ5-desaturase (Genbank accession number AB02931) (26% homology in 131 amino acids), and *M. alpina* Δ6-desaturase

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(accession number AF110510 (30% homology in 86 amino acids).
Since there was a reasonable degree of amino acid homology to
known desaturases, a full-length gene encoding a potential
desaturase was sought to determine its activity when expressed
5   in yeast.

                              Example III
           Isolation of the full length gene sequence from *T. aureum*
                              (ATCC 34304)
10

        To find the full-length gene, two separate PCR reactions
were carried out in an attempt to determine the two ends of
putative desaturase from the cDNA library.   For the 3' end of
the gene, RO898 (SEQ ID NO:7) (5'-
15   CCCAGTCACGACGTTGTAAAACGACGGCCAG-3') (designed based on the
sequence of the pBluescript SK(+) vector (Stragene, La Jolla,
CA) was used in a PCR amplification reaction along with a
gene specific primer RO930 (SEQ ID NO:8)(5'-
GACGATTAACAAGGTGATTTCCCAGGATGTC).   In this case, the Advantage
20   -GC cDNA PCR kit (Clonetech, Palo Alto, CA) was used to
overcome PCR amplification problems that occur with GC rich
sequences (61% for 1313 bp fragment).   PCR thermocycling
conditions were as follows: the template was initially
denatured at 94°C for 3 minutes, followed by 30 cycles of [94°C
25   for 30 seconds, 52°C for 30 seconds, and 72 °C for 1 minute],
and finally an extension cycle at 72 °C for 7 minutes with 20
pmoles of each primer.   The PCR products thus obtained was
resolved on a 1 %' agarose gel, excised, and gel purified using
the Qiagen Gel Extraction Kit (Qiagen, Valencia, CA).   The
30   staggered ends on the fragment was 'filled-in' using T4 DNA
polymerase (LifeTechnologies, Rockville, MD) as per
manufactures specifications and cloned into the PCR-Blunt
vector (Invitrogen, Carlsbad, CA) as described in Example II.
Clone 93-3 sequence overlapped the original 1313 bp fragment
35   and was found to contain an open reading frame, a stop codon,
and a poly A tail indicating that this was the 3' end of the
gene.   Two primers were designed based on clone 93-3 sequence
near the stop codon with an *XhoI* created site (underlined) as
follows: RO973 (SEQ ID NO:9) (5'-

                                46

GACTAACTCGAGTCACGTGGACCAGGCCGTGAGGTCCT-3') and RO974   (SEQ ID
NO:10) (5'-`GACTAACTCGAGTTGACGAGGTTTGTAT
GTTCGGCGGTTTGCTTG-3'). Two primers were deliberately chosen
because RO973, that contained the stop codon, was high in GC
5   (60%) and might not amplify well. On the other hand, RO974,
downstream of the stop codon, was much lower in GC (48%).

Following the same protocol as described above to isolate
the 5' end of the gene, RO899 (SEQ ID NO:11) (5'-
AGCGGATAACAATTTCACACAGGAAACAGC-3') (designed based on the
10   sequence of the pBluescript SK(+) vector) and the gene
specific oligonucleotide RO1004 (SEQ ID NO:12) (5'-
TGGCTACCGTCGTGCTGGATGCAAGTTCCG-3') were used for amplification
of the cDNA library. Amplification was carried out using 10
pmols of each primer and the cDNA polymerase kit (Clonetech,
15   Palo Alto, CA). The reaction conditions included an initial
denaturation at 94°C for 1 minute, followed by 30 cycles of
[94°C for 30 seconds, 68°C for 3 minutes], and finally an
extension cycle at 68°C for 5 minutes. The PCR products thus
obtained were cloned into the PCR-Blunt vector (Invitrogen,
20   Carlsbad, CA) following the same protocol as described above.
The recombinant plasmids were transformed into TOP10
supercompetent cells (Invitrogen, Carlsbad, CA), and clones
were sequenced. Clone 1004-5 contained an open reading frame,
several start codons, and overlapped the original 1313 bp
25   sequence indicating that this was the 5' end of the gene.

To isolate the full-length gene, a primer for the 5' end
of the putative desaturase was designed with a created EcoRI
(underlined) as follows: RO1046 (SEQ ID NO:13) (5'-
CGCATGGAATTCATGACGGTCGGGTTTGACGAAACGGTG-3').
30   To isolate a full-length clone, both RO1046/973 and
RO1046/974 were used with cDNA isolated from the library and
genomic DNA as a target. Both cDNA polymerase (Clonetech,
Palo Alto, CA) and -GC Advantage Polymerase (Clonetech, Palo
Alto, CA) were used to amplify their respective targets with
35   10 pmol of primer with the following reaction conditions: an
initial denaturation at 94°C for 1 minute, followed by 30
cycles of [94°C for 30 seconds, 68°C for 3 minutes], and
finally an extension cycle at 68°C for 5 minutes. The
reactions were gel purified, cut with EcoRI/XhoI and cloned

into *EcoRI/XhoI* prepared yeast expression vector pYX242
(Invitrogen, Carlsbad, CA) that had been treated with shrimp
alkaline phosphatase (Roche, Indianapolis, IN) to prevent
recircularization.   Initial analysis of the full-length

5   sequences showed several base changes. Clones 112-3 and 112-5
(designated pRTA7 and 8, respectively) were derived from the
amplification with genomic DNA and -GC Advantage polymerase
using primers RO1046/974. Clone 110-3 (designated pRTA5) was
derived from a reaction with RO1046/973, genomic DNA target

10   and cDNA polymerase. Clone 111-1 (designated pRTA6) was
isolated from the reaction using RO1046/974, cDNA target and -
GC Advantage polymerase kit. The sequence of these four
plasmids, pRTA5 (SEQ ID NO:14), pRTA6 (SEQ ID NO:15), pRTA7
(SEQ ID NO:16), pRTA8 (SEQ ID NO:17) is shown in Figures 3-6,

15   respectively.   (Plasmids pRTA7 and pRTA8 were deposited with
the American Type Culture Collection, 10801 University
Boulevard, Manassas, Virginia 20110 on April 19, 2001 and were
accorded accession numbers PTA-3301 and PTA-3300,
respectively.)   This putative desaturase of 1548 bp and 515

20   amino acids (see Figures 7-10 and SEQ ID NOS:18, 19, 20, 21,
respectively) had many of the characteristics of described
desaturases. The amino acids corresponding to the 5' end of
the enzyme are homologous to cytochrome b5. There are also
number of histidine boxes at the following amino acids: 178-

25   183- (Q)HDGSH (SEQ ID NO:59); 213-219- (Q)HVLGHH (SEQ ID
NO:60); 262-265- HPWH (SEQ ID NO:61); 271-275 -HKFQH (SEQ ID
NO:62); and 452-457(H)QIEHH (SEQ ID NO:63). At least either
an H or a Q precedes three of these histidine boxes which is
unusual. *Dictyostelium discoideum* (Genbank accession number

30   AB029311) has two similar boxes [(Q)HVIGHH (SEQ ID NO:64) and
(H)QVVHH] (SEQ ID NO:65), while *M. alpina* (Genbank accession
number AF067654) has (Q)HMLGHH (SEQ ID NO:66) and
Synechocystis sp. only has one (H)QVTHH (SEQ ID NO:67).

The sequences of the various putative desaturases differed

35   from each other. Several of the base changes resulted in a
change in amino acid, as shown in Table 1. These differences
could be naturally occurring variants, introduced by PCR
mismatch during final amplification, or a PCR error when the

initial cDNA was produced. There are 7 individual amino acid
changes between the four plasmids, none of which are shared
(see Figure 2A and B, underlined and bold amino acids). These
differences could alter the activity of the encoded enzyme.

5

Table 1

Amino Acid Differences in Different Clones

Amino Acid Number	PRTA5	PRTA6	PRTA7	PRTA8
99	F	S	F	F
280	F	F	L	F
284	F	F	F	S
317	Y	Y	N	Y
332	T	M	M	M
410	T	T	T	A
513	R	W	W	W

10   Example IV
                            Expression of Plasmids
                 Containing Putative Desaturases in Yeast

        All four plasmids were transformed into competent
15 *Saccharomyces cerevisiae* strain 334. Yeast transformation was
carried out using the Alkali-Cation Yeast Transformation Kit
(BIO 101, Vista, CA) according to conditions specified by the
manufacturer. Transformants were selected for leucine
auxotrophy on media lacking leucine (DOB [-Leu]). To detect
20 the specific desaturase activity of these clones,
transformants were grown in the presence of 50 μM specific
fatty acid substrates as listed below:
        a.   Linoleic acid (18:2n-6) (conversion to alpha-
              linolenic acid would indicate Δ15-desaturase
25   activity and conversion to gamma-linolenic acid
              would indicate Δ6-desaturase activity)
        b.   Alpha-linolenic acid (18:3n-3) (conversion to
              stearidonic acid would indicate Δ6-desaturase
              activity)
30   c.   Arachidonic acid (20:4n-6) (conversion to
              eicosapentaenoic acid would indicate Δ17-desaturase
              activity).
        d.   Adrenic acid (22:4n-6) (conversion to ω3-

docosapentaenoic acid would indicate Δ19-activity or conversion to ω6-docosapentaenoic acid would indicate Δ4-desaturase activity.

e.   ω3-Docosapentaenoic acid (22:5:n-3) (conversion to docosahexaenoic would indicate Δ4-desaturase activity.

The negative control strain was *S. cerevisiae* 334 containing the unaltered pYX242 vector, and these were grown simultaneously.

The cultures were vigorously agitated (250 rpm) and grown for 48 hours a 24°C in the presence of 50 μM (final concentration) of the various substrates in 50 ml of media lacking leucine after inoculation with overnight growth of single colonies in yeast peptone dextrose broth (YPD) at 30°C. The cells were pelleted, and the pellets vortexed in methanol; chloroform was added along with tritridecanoin (as an internal standard). These mixtures were incubated for at least an hour at room temperature or at 4°C overnight. The chloroform layer was extracted and filtered through a Whatman filter with 1 gm anhydrous sodium sulfate to remove particulates and residual water. The organic solvents were evaporated at 40°C under a stream of nitrogen. The extracted lipids were then derivitized to fatty acid methyl esters (FAME) for gas chromatography analysis (GC) by adding 2 mls of 0.5 N potassium hydroxide in methanol to a closed tube. The samples were heated to 95°C-100°C for 30 minutes and cooled to room temperature. Approximately 2 ml of 14% borontrifluoride in methanol was added and the heating repeated. After the extracted lipid mixture cooled, 2 ml of water and 1 ml of hexane were added to extract the fatty acid methyl esters (FAME) for analysis by GC. The percent conversion was calculated by dividing the product produced by the sum of (the product produced + the substrate added) and then multiplying by 100.

The results showed conversion of ω-3DPA to DHA and ADA to ω6-DPA. This would indicate Δ4-desaturase activity (see Table 2).

Table 2
Percent Conversion of Different Substrate Concentrations to Product

Clone	25 uM 22:4n-6	50 uM 22:4n-6	100 uM 22:4n-6	25 uM 22:5n-3	50 uM 22:5n-3	100 uM 22:5n-3
PYX242 (control)	0	0	0	0	0	1.28
PRTA5	3.91	0.9	1.24	10.	6.89	3.1
PRTA6	4.69	2.77	1.18	14.26	8.52	4.98
PRTA7	10.97	6.11	3.14	36.34	17.52	9.92
PRTA8	5.55	2.43	0.92	19.44	8.52	4.33

22:4n-6 to 22:5n-6 (Adrenic acid to ω6-Docosapentaenoic acid)
22:5n-3 to 22:6n-3 (ω3-Docosapentaenoic acid to Docosahexaenoic acid)

In particular, this is the first demonstration a Δ4-desaturase gene with *in vivo* expression data. The conversion for the four clones ranged from 3.91% to 10.97% for production of ω6-DPA from ADA and 10% to 36.34% for production of DHA from ω-3DPA. The enzyme appears to be much more active in the production of DHA rather than ω6-DPA, as indicated by the reduced percent conversion, 36.34% vs 10.97 %, respectively, for 25 μm of substrate for clone pRTA7. At 100 μm concentration of either substrate, the percent conversion (see Table 2) as well as the amount of product produced (data not shown) decreased, indicating that there may be feedback inhibition of the desaturation step by the substrate. The amount of ω3-DPA (22:5n-3) incorporated (as a percent of the total lipid) is similar for all four plasmids (see Table 3, below). However the amount produced as a percent of the total does vary from 2.74(PRTA5) to 8.11% (PRTA7). The difference in the conversion rates and percent produced could be due to the difference in sequence, hence amino acid variation of the encoded enzyme in the four plasmids.

Table 3

Fatty Acid as a Percentage of Total Lipid Extracted from Yeast

Clone	22:4(n-6) Incorporated	22:5(n-3) Produced	22:5(n-6) Incorporated	22:6(n-3) Produced
PYX242 (control)	38.96	0	11.2	0
PRTA5	14.5	0.59	19.8	2.74
PRTA6	16.07	0.79	17.97	4.38
PRTA7	39.88	4.91	14.21	8.11
PRTA8	36.94	2.17	17.45	4.25

25 μM substrate data shown

Key:

22:4(n-6)	=Adrenic acid
22:5(n-3)	=ω3-Docosapentaenoic acid
22:5(n-6)	=ω6-Docosapentaenoic acid
22:6(n-3)	=Docosahexaenoic acid

This data shows unequivocally that these plasmids indeed encode a Δ4-desaturase, which has preferred activity on conversion of ω3-DPA to DHA activity over conversion of ADA to ω6-DPA.

Example V
Expression of Δ4-desaturase with the
Mouse Elongase in Yeast

The plasmids pRTA7 and pRTA8 (which had the two highest percent conversion) may be individually co-transformed with pRMELO4, a clone that contains a mouse elongase gene from pRAE-84 (see U.S. Patent Application Serial No. 09/624,670 incorporated herein in its entirety by reference). The mouse elongase of 879 base pairs (see Figure 11 (SEQ ID NO:22) and Figure 12 (SEQ ID NO:23)) may be cloned as an EcoRI/SalI fragment in the yeast expression vector pYES2 (Invitrogen, Carlsbad, CA) at the EcoRI/XhoI sites. This elongase of 292 amino acids catalyzes several of the elongation steps in the PUFA pathway, specifically AA to ADA and EPA to ω3-DPA. ADA and ω3-DPA are substrates for the Δ4-desaturase. Yeast transformants may be selected on minimal media lacking leucine

52

and uracil (DOB[-Leu-Ura]) for selection of Δ4-desaturase

(pRTA7 or pRTA8) and pRMELO4 (mouse elongase). Growth and

expression of the yeast culture containing pRMELO4 and pRTA7

or pRTA8 in minimal media lacking uracil and leucine and 2%

5 galactose may result in elongation of exogenously added AA to

ADA and Δ4 desaturation to ω6-DPA. Additionally,

supplementation of EPA to the yeast minimal media may result

in elongation to ω3-DPA by the elongase which may then be

desaturated by the Δ4-desaturase to produce DHA as shown in

10 Figure 1. This has been previously demonstrated with

elongases and other desaturases to produce AA and EPA (see PCT

application WO 00/12720) and provides parallel experimental

data to show that elongation of a substrate and subsequent

desaturation can take place _in vivo_ in an organism such as

15 yeast and potentially other organisms. Further, the present

data demonstrates the ability of the Δ4-desaturase to work

with another enzyme in the PUFA biosynthetic pathway to

produce either ω6-DPA or DHA from the precursors AA and EPA,

respectively.

20

<div align="center">

Example VI

Homologue of Δ4-desaturase from _Schizochytrium aggregatum_
(ATCC 28209)

</div>

25   In parallel to Example II, RNA was isolated by the acid

phenol method from _Schizochytrium aggregatum_ (_S. aggregatum_)

ATCC 28209. Briefly, pellets of _S. aggregatum_ were washed with

cold deionized water and repelleted for 5 minutes at 3000 rpm.

Approximatley 10 ml of TES solution (10 mM Tris-CL pH 7.5, 10

30 mM EDTA, and 0.5% SDS) was used to resuspend the pellet. Then

10 ml of acid phenol was added and incubation followed for one

hour at 65° C. The pellet was placed on ice for 5 minutes,

centrifuged for 5 minutes at 1000 x g at 4° C, and the aqueous

phase transferred to a new tube. An additional 10 ml of acid

35 phenol was added to the aqueous phase, the mixture vortexed

and separated as before. The aqueous phase containing the

nucleic acids was transferred to a new tube. Approximately

1ml of sodium acetate pH 5.3 and 25 ml of ice-cold ethanol

were added for overnight precipitation at -70 C. The next

<div align="center">53</div>

day, the tubes were centrifuged for 15 minutes at 14,000 rpm
at 4° C and the supernatant decanted. The pellet was washed
with 10 ml of 70% ethanol and centrifuged as in the previous
step. The pellet was dried and resuspended in 500ul of RNAse
5   free deionized water. The RNA was further purified using the
Qiagen RNeasy Maxikit (Qiagen, Valencia, CA) as per the
manufacturer's protocol.

        cDNA was generated using oligo dT with the SuperScript
Preamplification system (Life Technologies, Rockville, MD)
10   with 5 ug of RNA from *S. aggregatum*. Since *S. aggregatum*
produces large quantities of DHA, a Δ4-desaturase would be
required for DHA production. In an identical experiment,
primers RO753 (SEQ ID NO:5) and RO754 (SEQ ID NO:6) were used
in the same reaction as in Example II to produce a band around
15   800 base pairs. As before the DNA generated from the PCR
reaction was separated on a 1% gel, excised, purified, and
cloned into the PCR-Blunt vector (Invitrogen, Carlsbad, CA).
The DNA sequence generated from clones saa9 and saa5
overlapped to create the sequence saa.con (SEQ ID NO:24 and
20   Figure 13). The translation of the open reading frame of
saa.con DNA sequence to an amino acid sequence (SEQ ID NO:25
and Figure 14) aligned with pRTA7 is shown in Figure 15. The
amino acid sequence of the Δ4-desaturase from clone pRTA7 has
79.1% identity with the translated saa.con sequence over 249
25   amino acids. This sequence, due to its high identity with a
known Δ4-desaturase, is most likely a fragment of a Δ4-
desaturase from *S. aggregatum*. This example provides evidence
that this procedure can be used to isolate Δ4-desaturases from
other organisms.

30

                            Example VII
          Isolation of Δ4-Desaturase Nucleotide Sequences from
      Schizochytrium aggregatum (ATCC 28209) and *Thraustochytrium*
                          aureum (BICC 7091)
35

        To isolate the 5' and 3'-ends, new primers were designed
based on the internal sequence of the isolated *S. aggregatum*
fragment shown in Example VI. For the 5 prime end of the gene,
RO1240 (SEQ ID NO:26) (5'-CCC TCG ATG ATG TGG TTG ACG ATG AAC

-3') was used and subsequently 5 prime nested primer RO1239

(SEQ ID NO:27) (5'-CGG AGC ATG GGG TAG GTG CTG AAG AC-3'). For

the 3 prime end, RO1236 (SEQ ID NO:28) (5'-CCA ACT GCC GTT ACG

CCA GCA AGT -3') was used followed by 3 prime nested primer

5   RO1237 (SEQ ID NO:29) (5'-CAA GCT CTT CTT CAT CGC CCA CTT TTC

G-3'), for a second reaction to isolate the other end of the

gene. RACE (rapid amplification of cDNA ends) ready cDNA was

used as a target for the reactions. To prepare this material,

approximately 5 μg of total RNA was used according the

10   manufacturer's direction with the GeneRacer™ kit (Invitrogen,

Carlsbad, CA) and Superscript II™ enzyme (Invitrogen,

Carlsbad, CA) for reverse transcription to produce cDNA

target. For the initial amplification of the ends, the

following thermocycling protocol was used in a Perkin Elmer

15   9600: initial melt at 94°C for 2 minutes followed by 5 cycles

of 94°C for 30 seconds and 72°C for 3 minutes, 10 cycles of

94°C 30 seconds, 70°C for 30 seconds, and 72°C for 3 minutes

and 20 cycles of 94°C for 30 seconds, 68°C for 30 seconds and

72°C for 3 minutes, followed by an extension of 72°C for 10

20   minutes. The first PCR reaction was performed with 10 pMol of

RO1240 and 30 pMol GeneRacer™ 5 prime primer (SEQ ID NO:30)

(5'- CGA CTG GAG CAC GAG GAC ACT GA-3') or RO1236 and

GeneRacer™ 3 prime primer (SEQ ID NO:31) (5'- GCT GTC AAC GAT

ACG CTA CGT AAC G-3'). The reaction contained 1 ul of cDNA in

25   a final volume of 50 ul with Platimum Taq™ PCRx (Clonetech,

Palo Alto, CA) using MgSO₄ according to the manufacturer's

directions. A nested reaction was performed with 1 ul of the

initial reaction, 10 pmol of nested primer RO1239 and 30 pmol

of the GeneRacer™ nested 5 prime primer (SEQ ID NO:32) (5'-

30   GGA CAC TGA CAT GGA CTG AAG GAG TA-3') or GeneRacer™ nested 3

prime primer (SEQ ID NO:33) (5'- CGC TAC GTA ACG GCA TGA CAG

TG -3') and nested primer RO1237 using the same conditions as

the first reaction. Agarose gel analysis of the PCR products

showed a band around 800 base pairs for the 5 prime reaction
and approximately 600 base pairs for the 3 prime reaction.
Subsequent cloning into pCR Blunt (Invitrogen, Carlsbad, CA),
transformation into Top10 competent cells (Invitrogen,

5   Carlsbad, CA), and sequencing revealed an open reading frame
with both a start and stop codon. Primers RO1241 (SEQ ID
NO:34) (5'-GAT ATC GAA TTC ATG ACG GTG GGC GGC GAT GAG G-3')
and RO1242 (SEQ ID NO:35) (5'-GTA CTT AAG CTT TCA CTT GGA CTT
GGG GTG GTC C-3') with restrictions sites added for cloning

10   (see underlined EcoRI, and HindIII respectively) were used to
isolate a full length gene. As shown above, 10 pmol of
primers RO1241 and 1242 were used with Platimum Taq™ PCRx
(Clonetech, PaloAlto, CA) using MgSO₄ according to the
manufacturer's protocol with 2 ul of the cDNA as target. The

15   thermocycling parameters were as follows: initial melt at 94°C
for 2 minutes followed by 5 cycles of 94°C for 30 seconds and
72°C for 2 minutes, 5 cycles of 94°C 30 seconds, 70°C for 2
minutes and 20 cycles of 94°C for 30 seconds, 65°C for 30
seconds and 68°C for 2 minutes, followed by an extension of

20   68°C for 10 minutes. The large product of the reaction was
gel purified using the QiaQuick gel purification kit (Qiagen,
Valencia, CA) cut with EcoRI and HindIII and ligated to pYX242
EcoRI/HindIII linearized DNA with the Rapid ligation kit
(Roche, Indianapolis, IN) and designated pRSA-1. The clone

25   pRSA1 contained a full length gene of 1530 bp (SEQ ID NO:36,
Figure 16) and an open reading frame of 509 amino acids (SEQ
IN NO:37, Figure 17). (Plasmid pRSA-1 was deposited with the
American Type Culture Collection, 10801 University Boulevard,
Manassas, Virginia 20110 on March 27, 2002 and was accorded

30   accession number PTA-4186.)

The second Δ4-desaturase was identified by a partial
sequence isolated using the primer combination of RO1201 (SEQ
ID NO:38) (5'-CGT GTT CGC TGC CTT TGT CGG AAC TTG CAT CC- 3'
and RO1202 (SEQ ID NO:39) (5' - TTG ACA ATA AAC ATG GAG GCG

35   AGG ACC TCT CCG- 3') based on the sequence of pRTA7 (SEQ IN

NO:16) as described in Example III. The genomic DNA (gDNA),
was prepared as described in Example I, from *Thraustochytrium*
aureum (BICC 7091) (Biocon India Ltd., Bangalore, India). PCR
amplification was carried out in a 100 µl volume containing:
5   5 µl of isolated T7091 gDNA, 1.0 U of cDNA Polymerase
(Clonetech, PaloAlto, CA) and 10 pMol of primers according the
manufacturer's protocol. Thermocycler conditions in Perkin
Elmer 9600 were as follows: 94°C for 3 min, then 35 cycles of
94 °C for 30 sec., 60°C for 30 sec., and 72°C for 1 min. PCR
10 was followed by an additional extension at 72°C for 7 minutes.
A 600 bp fragment was gel purified, ends filled-in using T4
DNA Polymerase (LifeTechnologies, Rockville, MD, cloned into
the pCR-Blunt vector (Invitrogen, Co., Carlsbad, CA), and the
recombinant plasmids transformed into TOP10 supercompetent
15 cells (Invitrogen, Carlsbad, CA). Sequencing of the clones
revealed high homology with pRTA7 (82.1% in 196 amino acids).

    For isolation of a full-length gene, a cDNA library was
constructed with mRNA isolated from total RNA using oligo dT
20 cellulose resin. The pBluescript II XR library construction
kit (Stratagene, La Jolla, CA) was then used to synthesize
double stranded cDNA which was then directionally cloned
(5'*NotI*/3'*EcoRI*) into pBluescript II KS(+) vector. The *T.*
aureum (BICC 7091) library contained approximately 1.89 x 10^8
25 clones, each with an average insert size, of approximately 1300
bp.
    Primers RO1210 (SEQ ID NO:40) (5'-GCT GGT TGG ACT TTG GAC
ATG ATT GGA TCC- 3') and RO1211 (SEQ ID NO:41) (5'-TAC ATT GGC
AGG CCA ACC ATG TAG AGA ACG – 3') were designed to amplify 5'
30 and 3' sequences, respectively. RO1210/RO899 (SEQ ID NO:11)
and RO1211/RO898(SEQ ID NO:7) were set up with cDNA Polymerase
(Clonetech, PaloAlto, CA), 5 ul of cDNA from the library under
the same conditions as described for isolating the original
fragment earlier in this example. After cloning and
35 sequencing of fragments an additional internal primer RO1214
(SEQ ID NO:42) (5'-GGA TTC AAT CAT GTC CAA AGT CCA ACC AGC-3')

with RO898 from the vector was used to identify the 5' end of
the gene. In a 50 μl reaction, 10 pmol of each primer with
5ul of library DNA as target with Platimum Taq™ PCRx
(Clonetech, PaloAlto, CA) with MgSO₄ was used according to the

5　manufacturer's protocol. The cycling protocol was as follows:
an initial melt of 94°C for 5 minutes followed by 35 cycles of
94°C for 45 seconds, 55°C for 30 seconds, 68°C for 2 minutes
and an extension cycle of 72°C for 7 minutes.

　　　The full-length Δ4-desaturase from *T. aureum* (BICC 7091)

10　was isolated with 5' primer RO1223 (SEQ ID NO:43) (5'-TCT GAT
GAA TTC ATG ACG GCC GGA TTT GAA GAA G-3') and 3' primer RO1224
(SEQ ID NO:44) (5'-GTC TAG CTC GAG TTA GTT CTT GTC CCA GGC AGG
CA-3') with added restriction sites EcoRI and XhoI
(underlined), respectively, added for cloning purposes. In a

15　50 μl reaction, 10 pmol of each primer, with 5 ul of library
DNA as target, with Platimum Taq™ PCRx (Clonetech, PaloAlto,
CA) with MgSO₄ according to the manufacturer's protocol, were
used. The cycling protocol was as follows: an initial melt of
94°C for 5 minutes followed by 35 cycles of 94°C for 45

20　seconds, 55°C for 30 seconds, 68°C for 2 minutes and an
extension cycle of 72°C for 7 minutes. The single band was
separated on an agarose gel, purified, cut with EcoRI and
XhoI, and ligated to pYX242 linearized with the same enzymes.
Sequence analysis of the full-length clone designated, pRTA11

25　(see Figure 18) (SEQ ID NO:45) revealed an open reading frame
of 1542 base pairs encoding a protein of 513 amino acids (see
Figure 19) (SEQ ID NO:46). (Plasmid pRTA11 was deposited with
the American Type Culture Collection, 10801 University
Boulevard, Manassas, Virginia 20110 on March 27, 2002 and was

30　accorded accession numbers PTA-4187.)

　　　　　　　　　　　　　　Example VIII
　　　　　　　　　　　　Expression of Putative
　　　　　　　Δ4-Desaturases pRSA1 and pRTA11 in Yeast

35
　　　Both plasmids were transformed into competent
Saccharomyces cerevisiae strain 334 and grown as described in
Example IV with either 50 μM ADA or ω3-DPA. As shown in Table
4, both ω6-DPA and DHA were produced when 334 (pRSA1) or

(pRTA11) was grown with ADA or ω3-DPA, which are the products of a Δ4-desaturation.

Table 4
Fatty Acid as a Percentage of
Total Lipid Extracted from Yeast

Clone	22:4(n-6) Incorpor-ated	22:5(n-6) Produced	22:5(n-3) Incorpor-ated	22:6(n-3) Produced
PYX242 (control)	15.03	0	20.46	0.25
PYX242 (control)	55.36	0	62.98	0.42
PRTA11	50.73	5.42	42.39	9.17

Key:

22:4(n-6)	=Adrenic acid
22:5(n-3)	=ω3-Docosapentaenoic acid
22:5(n-6)	=ω6-Docosapentaenoic acid
22:6(n-3)	=Docosahexaenoic acid

When the percent conversion of the substrate to product was calculated as described in Table 5, the preferred substrate, by virtue of the higher percent conversion, was the ω3-DPA to produce DHA. This data shows clearly that these plasmids also encode Δ4-desaturases.

Table 5
Percent Conversion of Two Substrates to Product

Clone	50 uM 22:4n-6	50 uM 22:5n-3
PYX242 (control)	0	1.2
PRSA1	3.64	9.7
PYX242 (control)	0	0.66
PRTA11	9.65	17.78

22:4n-6 to 22:5n-6 (Adrenic acid to ω6-Docosapentaenoic acid)
22:5n-3 to 22:6n-3 (ω3-Docosapentaenoic acid to Docosahexaenoic acid)

Example IX

Demonstration of Co-expression of a Δ4-desaturase with a Mouse
                        Elongase in Yeast

    As described in Example V, the *T.aureum* (ATCC 34304) Δ4-
5. desaturase was co-transformed with the mouse elongase pRMELO4
    (recloned from the plasmid pRAE84 into pYES2).

    Table 6 shows that when 10 μM of the substrate EPA
    (20:5n-3) was added, the elongase was able to add two carbons
    to EPA to produce ω3-DPA, and the desaturase converted ω3-DPA
10 to DHA. No DHA was produced by the control transformation
    334(pYX242/pYES2). A small amount of ω3-DPA was seen in the
    control, but was a contaminant of the added substrate EPA.
    Thus, *T. aureum* Δ4-desaturase was able to produce a product in
    a heterologous expression system that was the product of
15 another heterologous enzyme (the mouse elongase) from the PUFA
    biosynthetic pathway to produce the expected PUFA. This
    demonstrates that Δ4-desaturase can indeed work with other
    heterologous enzymes in the PUFA pathway in a heterologous
    expression system such as yeast.
20

                              Table 6
            Fatty Acid (μg) Extracted Lipid from Yeast

Clone	EPA Incorporated	ω3-DPA Produced by elongase	DHA Produced by desaturase
PYX242/ PYES2 (control)	59.38	2.54	0
PRTA7/ PRMELO4 (mouse elongase)	47.04	14.76	1.55

25
    10 μM substrate added

Example IX
Isolation of a Novel Desaturase Gene from the Algae *Isochrysis*
galbana (CCMP1323)

5   The fatty acid composition of the algae *Isochrysis*
galbana (*I. galbana*) (CCMP 1323) was investigated to determine
the polyunsaturated fatty acids (PUFAs) produced by this
organism. This algae showed a substantial amount of long
chain PUFA including omega 3-docosapentaenoic acid (omega 3-
10   DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). In
fact DHA was present in the highest amount representing 19% of
the total lipid. Thus, *I. galbana* was predicted to possess a
Δ4-desaturase capable of converting omega 3-DPA to DHA. The
goal was therefore to isolate the predicted Δ4-desaturase gene
15   from *I. galbana*, and to verify the functionality of the enzyme
by expression in an alternate host.

          Frozen pellets of *I. galbana* were obtained from
Provasoli-Guillard National Center for Culture of Marine
Phytoplankton (CCMP, West Boothbay Harbor, ME). These pellets
20   were crushed in liquid nitrogen and total RNA was extracted
from *I. galbana* by using the Qiagen RNeasy Maxi Kit (Qiagen,
as per manufacturers instructions. From this total RNA, mRNA
was isolated using oligo dT cellulose resin, which was then
used for the construction of a cDNA library using the
25   pBluescript II XR library construction kit (Stratagene, La
Jolla, CA). The cDNA thus produced was directionally cloned
(5'*NotI*/3'*EcoRI*) into pBluescript II KS (+) vector. The *I.*
galbana library contained approximately 9.4×10^4 clones per
μl, each with an average insert size of approximately 1300 bp.
30   Two thousand primary clones from this library were sequenced
from the 5' end using the M13 forward primer (SEQ NO ID:47)
(5'-AGC GGA TAA CAA TTT CAC ACA GG-3'). Sequencing was
carried out using the ABI BigDye sequencing kit (Applied
Biosystems, CA) and the MegaBase Capillary DNA sequencer
35   (Amersham biosciences, Piscataway, NJ).

A 647 bp clone containing the 3' end of this novel Δ4-
desaturase designated 'iso25-A09' was obtained from sequencing
of the 2000 library clones. This fragment shared ~30% amino
acid sequence identity with other known delta 5 and delta 6

5   desaturases. Since this fragment did not contain the stop
codon of the gene, additional clones containing the 3' end of
this gene were obtained by PCR amplification of the cDNA
library (template) using the 3'-end vector primer RO899 (SEQ
ID NO:11) and RO1270 (SEQ ID NO:48) (5'- CAC CTG GCT CGA GTC

10   GAC GAT GAT GG -3'). PCR amplification was carried out using
Platinum Taq (HF) DNA polymerase (Invitrogen, Carlsbad, CA).
Amplification was carried out in a 50 µl total volume
containing: 1 µl of the cDNA library ligation mixture, PCR
buffer containing 20 mM Tris-Cl, pH 8.4, 50 mM KCl (final

15   concentration), 200 µM each deoxyribonucleotide triphosphate,
10 pmole of each primer, 1.5 mM MgSO$_4$, and 0.5 µl of Platinum
Taq (HF) DNA polymerase. Amplification was carried out as
follows using the Perkin Elmer 9600 machine: initial melt at
94°C for 2 minutes followed by 5 cycles of 94°C for 30 seconds

20   and 72°C for 3 minutes, 10 cycles of 94°C 30 seconds, 70°C for
30 seconds, and 72°C for 3 minutes and 20 cycles of 94°C for 30
seconds, 68°C for 30 seconds and 72°C for 3 minutes, followed
by an extension of 72°C for 10 minutes. From this
amplification no bands were visible which might have been due

25   to the low amounts of this gene in the library. Thus 2 µl of
this PCR reaction was used as a template for a second PCR
reaction involving Platinum Taq (HF) DNA polymerase under that
same PCR components as described above. However, this time
amplification was carried out as follows: initial denaturation

30   at 94°C for 3 minute, followed by 30 cycles of the following:
94°C for 45 sec, 55°C for 30 sec, 68°C for 2 min. The reaction
was terminated at 4°C. A 670 bp PCR band was thus obtained
which was gel purified, and cloned into PCR-Blunt vector
(Invitrogen, Carlsbad, CA). The recombinant plasmids were

transformed into TOP10 supercompetent cells (Invitrogen,
Carlsbad, CA), and clones were sequenced and analyzed. Clones
'iso25-A09-6' and iso25-A09-1' were thus obtained that
contained the 3' end of the gene along with the 'TAA' stop
5   codon and the poly-A tail. This clone did overlap with the
original 'iso25-A09' fragment.

To isolate the 5' end of this gene, RACE (rapid
amplification of cDNA ends) ready cDNA was used as a target
for the reactions. To prepare this material, approximately 5
10   µg of total RNA was used according the manufacturer's
direction with the GeneRacer™ kit (Invitrogen, Carlsbad, CA)
and Superscript II™ enzyme (Invitrogen, Carlsbad, CA) for
reverse transcription to produce cDNA target. This cDNA was
then use as a template for a PCR reaction involving 30 pmol
15   GeneRacer™ 5' primer (SEQ ID NO:30) (5'- CGA CTG GAG CAC GAG
GAC ACT GA-3') in combination with 10 pmols of any one of the
following gene-specific primers:
RO1286 (SEQ ID NO:49)
5'- CGT ACC CGG TGC AAT AGA AGG TGA G -3'
20   RO1287 (SEQ ID NO:50)
5'- CCA TCA TCG TCG ACT CGA GCC AGG TG -3'
RO1288 (SEQ ID NO:51)
5'- TGT GGA GCC ATG TGG TGC TCG ATC TG -3'
PCR amplification was carried out using Platinum Taq DNA
25   polymerase (Invitrogen, Carlsbad, CA) in a 50 µl total volume
containing: 1 µl of the RACE-cDNA, PCR buffer containing 20 mM
Tris-Cl, pH 8.4, 50 mM KCl (final concentration), 200 µM each
deoxyribonucleotide triphosphate, 1.5 mM MgSO$_4$, and 0.5 µl of
Platinum Taq DNA polymerase. Amplification was carried out as
30   follows using the Perkin Elmer 9600 machine: initial melt at
94°C for 2 minutes followed by 5 cycles of 94°C for 30 seconds
and 72°C for 3 minutes, 10 cycles of 94°C 30 seconds, 70°C for
30 seconds, and 72°C for 3 minutes and 20 cycles of 94°C for 30
seconds, 68°C for 30 seconds and 72°C for 3 minutes, followed

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by an extension of 72°C for 10 minutes. All these primer
combinations resulted in bands, which were gel purified,
filled-in with T4-DNA polymerase, cloned into PCR-blunt vector
and transformed into TOP10 supercompetent cells. Sequencing
of these clones like 'iso25-A09-33-5', 'iso25-A09-31-3',
iso25-A09-30-1' and iso25-A09-32-3' revealed the 5' end of
this gene containing the 'ATG' start site, the cytochrome b5
domain and two histidine boxes. These clones overlapped each
other and also overlapped the original 'iso25-A09' fragment
that contained the third histidine box.

    To isolate the full length of this gene both genomic DNA,
as well as cDNA obtained (from RACE), were used as templates
in PCR reactions with the following primers:
RO 1400 (SEQ ID NO:52)
5'- TCA ACA GAA TTC ATG TGC AAC GCG GCG CAG GTC GAG ACG CAG -
3'
(This forward primer contained an EcoRI site (underlined)
along with the 'ATG' start site (bold) suitable for cloning
into the yeast expression vector pYX242).

RO 1401 (SEQ ID NO:53)
5'- AAA AGA AAG CTT TTA GTC CGC CTT GAC CGT GTC GAC CAA AGC -
3'
(This reverse primer contained a HindIII site (underlined)
along with the 'TAA' stop site (bold) for cloning into
pYX242). PCR amplification was carried out using Advantage-GC
cDNA polymerase (Clonetech, Palo Alto, CA) in a 50 μl total
volume containing: 1 μl of the RACE-cDNA or 2 μl of genomic
DNA, PCR buffer containing [40 mM Tricine-KOH pH 9.2, 15 mM
KOAc (final concentration), 3.5 mM Mg(OAc)$_2$, 5% DMSO, 3.75
μg/ml BSA, 200 μM each deoxyribonucleotide triphosphate, 1M
GC-melt, and 1 μl of Advantage-GC cDNA polymerase. The
thermocycling protocol included an initial denaturation at
94°C for 1 min, followed by 30 cycles of the following

[denaturation at 94°C for 30 seconds and annealing at 68°C for 3 minutes], a final extension at 68°C for 5 minutes, followed by termination at 4°C.

5   A ~1.35 kb band was obtained which was gel purified, digested with the restriction enzymes *EcoRI/HindIII* for 2 hours, cleaned through the QiaQuick PCR purification kit (Qiagen, Valencia, CA), and cloned into the pYX242 yeast expression vector (Novagen, Madison, WI) previously digested

10   with *EcoRI/HindIII*. This construct was labeled pRIG6 and consisted of the 'iso25-A09' full length gene isolated from RACE-derived cDNA and the pYX242 vector. This was transformed into yeast SC334 for expression studies.

       The full length gene of 'iso25-A09' present in pRIG6 was

15   1302 bp in length (SEQ ID NO:54) (Figure 20) and encoded a protein of 433 amino acids (SEQ ID NO:55) (Figure 21). A tFastA search of the deduced protein sequence of this gene showed the protein to have 30.6% identity with the Δ5-desaturase from *I. galbana* (U.S. Patent Appln. No. 10/054,534

20   incorporated in its entirety by reference). Also the predicted protein of this gene was 30.8% identical to the Δ4-desaturase from *Thraustochytrium aureum* (ATCC 34304)(Figure 22). (Further, the DNA sequence of the gene was found to exhibit 42.37% sequence identity to the nucleotide sequence

25   encoding the *T. aureum* (ATCC 34304)) Δ4-desaturase, 43% identity to the nucleotide sequence encoding the *S. aggregatum* (ATCC 28209) Δ4-desaturase, and 39.7% identity to the nucleotide sequence encoding the *T. aureum* (BICC7091) Δ4-desaturase sequence.) Like all front-end desaturating enzyme

30   genes like Δ5- and Δ6-desaturase, this gene contains a cytochrome b5 domain within the 5'-end of its sequence. This cytochrome b5 is though to function as the immediate electron donor for the desaturases, and functions in a number of oxidation-reduction reactions involving NADH-dependent

35   desaturation. This gene also possessed the three histidine-

rich motifs that are present in all membrane-bound

desaturases. These are present at position 153 to 158 (HMGGH)

(SEQ ID NO:71), 188 to 193 (HNKHH) (SEQ ID NO:72), and 347 to

352 (QIEHH) (SEQ ID NO:73). These histidine-rich boxes are

5   believed to co-ordinate the diiron-oxo structure at the

enzyme's active site, and are necessary for enzyme activity.

These features are consistent with this gene product being a

member of the membrane-bound desaturase/hydroxylase family of

the diiron-oxo proteins (3) and also being a front-end

10   desaturating enzyme. The G+C content of this gene is 64.2%.

Example X
Expression of pRIG6, a Novel Desaturase from *Isochrysis*
galbana (CCMP 1323), in Yeast

15

To determine the substrate specificity and the class of

reaction catalyzed by a novel desaturase from *I. galbana*, the

pRIG6 construct was heterologously expressed in a

Saccharomyces cerevisiae (SC334), as described below. Since

20   *S. cerevisiae* cannot synthesize fatty acids beyond oleic acid

(OA, 18:1 n-9), it is an ideal system to use to determine

enzyme activity on substrates longer than OA since no

background enzyme activity will be detected. Here, substrates

can be exogenously supplied to the host, taken up by the cell

25   and acted on by the expressed protein of the transformed gene.

Clone pRIG6, which consisted of the full-length 'iso25-

A09' desaturase from *I. galbana* cloned into pYX242, was

transformed into *Saccharomyces cerevisiae* (SC334) using the

Alkali-Cation Yeast Transformation kit (BIO 101, Vista, CA).

30   (Plasmid pRIG6 was deposited with the American Type Culture

Collection, 10801 University Boulevard, Manassas, Virginia

20110 on April 5, 2002 and was accorded accession number PTA-

4209.) Transformants were selected for leucine auxotrophy on

media lacking leucine (DOB [-Leu]). To detect the specific

35   desaturase activity of these clones, transformants were grown

in the presence of 50 µM specific fatty acid substrates as
listed below:

a.   Linoleic acid (LA, 18:2n-6) - conversion to α-linolenic
5   acid (ALA, 18:3n-3) indicates Δ15- desaturase activity;
conversion to gamma-linolenic acid indicates Δ6-
desaturase activity.

b.   Dihomo-gamma-linolenic acid (20:3n-6)- conversion to
eicosatetraenoic acid (ETA, 20:4n-3) indicates Δ17-
10   desaturase activity; conversion to arachidonic acid (ARA,
20:4n-6) indicates Δ5-desaturase activity.

c.   Omega-6-eicosadienoic acid (20:2n-6)-conversion to
Dihomo-gamma-linolenic acid (20:3n-6) indicates Δ8-
desaturase activity.

15 d.   Adrenic acid (22:4n-6)-conversion to ω6-docosapentaenoic
acid (22:5n-6) indicates Δ4- desaturase activity.

e.   Omega 3-docosapentaenoic acid (22:5n-3)-conversion to
Docosahexaenoic acid (22:6n-3) indicates Δ4-desaturase
activity.

20

The negative control strain consisted of *S. cerevisiae*
transformed with the pYX242 vector, and these cultures were
grown simultaneously and analyzed.

The cultures were vigorously agitated (250 rpm) and grown
25 for 48 hours a 24°C in the presence of 50 µM (final
concentration) of the various substrates (Table 7). The cells
were spun down, washed once in distilled water, and the
pellets vortexed in methanol; chloroform was added along with
tridecanoin (as an internal standard). These mixtures were
30 incubated for at least an hour at room temperature, or at 4°C
overnight. The chloroform layer was extracted and filtered
through a Whatman filter with 1 gm anhydrous sodium sulfate to
remove particulates and residual water. The organic solvents
were evaporated at 40°C under a stream of nitrogen. The
35 extracted lipids were then derivitized to fatty acid methyl

esters (FAME) for gas chromatography analysis (GC) by adding 2
ml 0.5 N potassium hydroxide in methanol to a closed tube.
The samples were heated to 95°C-100°C for 30 minutes and cooled
to room temperature. Approximately 2 ml 14% borontrifluoride
5 in methanol was added and the heating repeated. After the
extracted lipid mixture cooled, 2 ml of water and 1 ml of
hexane were added to extract the FAME for analysis by GC. The
percent conversion was calculated using the formula:

10 % Conversion =   [% Product]   X 100
                        [% Product + % Substrate]

Table 7 shows the substrate specificity of the novel
desaturase expressed in yeast. Here, the expressed pRIG6
15 clone was capable of converting 15.3% of ω3-
docosapentaenoic acid (22:5n-3) to docosahexaenoic acid
(22:5 n-3), indicating that the gene was a Δ4-
desaturase. In addition, this enzyme was capable of
converting 11% of adrenic acid (22:4n-6) to ω6-
20 docosapentaenoic acid (22:5n-6), which also indicated
Δ4-desaturase activity.
     The fatty acids of interest are represented as a
percentage of the total lipids extracted from yeast. GC/MS
was employed to identify the products. Under these
25 conditions, the clones did not exhibit other desaturase
activities. This confirmed the gene isolated to be a novel
Δ4-desaturase gene. No background substrate conversion was
detected with using just the vector alone. This data
indicates that this novel Δ4-desaturase can be expressed in a
30 heterologous system and would thus be useful in the production
of transgenic oil containing DHA.

Table 7

<u>Isochrysis galbana (CCMP 1323) Delta 4-Desaturase Expression</u>
<u>in Baker's Yeast at 24°C</u>

Clone	Desaturase activity	Substrate* Incorpor.	Substrate Produced	% Conversion of Substrate
PRIG6	Δ6	LA (8.35%)	GLA (0%)	0
(pYX242 +	Δ5	DGLA (16.34%)	AA (0.29%)	0
Delta 4	Δ8	ω6-EDA (19.53%)	DGLA (0%)	0
	Δ4	ADA (23.93%)	ω6-DPA (3.15%)	11%
	Δ4	ω3-DPA (32.57%)	DHA (5.89%)	15.3%
Control	Δ6	LA (9.18%)	GLA (0%)	0
(pYX242)	Δ5	DGLA (10.5%)	AA (0%)	0
	Δ8	ω6-EDA (16.56%)	DGLA (0%)	0
	Δ4	ADA (15.55%)	ω6-DPA (0%)	0
	Δ4	ω3-DPA (26.03%)	DHA (0.29%)	0

* 50 μM substrate used

Numbers in parenthesis represent fatty acid as a percentage of total lipids from yeast

Key:

LA= Linoleic acid (18:2n-6)

GLA= Gamma-linolenic acid (18:3n-6)

DGLA= Dihomo-gamma-linolenic acid (20:3n-6)

AA= Arachidonic acid (20:4n-6)

ω6-EDA= omega-6 Eicosadienoic acid (20:2n-6)

ADA= Adrenic acid (22:4n-6)

ω3-DPA= omega-3 Docosapentaenoic acid (22:5n-6)

ω6-DPA= omega-6 Docosapentaenoic acid (22:5n-3)

DHA= Docosahexaenoic acid (22:6n-3)

Nutritional Compositions

The PUFAs described in the Detailed Description may
be utilized in various nutritional supplements, infant
formulations, nutritional substitutes and other
nutritional solutions.

I. INFANT FORMULATIONS

A.   Isomil® Soy Formula with Iron:

Usage: As a beverage for infants, children and adults with an
allergy or sensitivity to cows milk. A feeding for patients
with disorders for which lactose should be avoided: lactase
deficiency, lactose intolerance and galactosemia.

Features:

    -Soy protein isolate to avoid symptoms of cow's-milk-
    protein allergy or sensitivity.
    -Lactose-free formulation to avoid lactose-associated
    diarrhea.
    -Low osmolality (240 mOs/kg water) to reduce risk of
    osmotic diarrhea.
    -Dual carbohydrates (corn syrup and sucrose) designed to
    enhance carbohydrate absorption and reduce the risk of
    exceeding the absorptive capacity of the damaged gut.
    -1.8 mg of Iron (as ferrous sulfate) per 100 Calories to
    help prevent iron deficiency.
    -Recommended levels of vitamins and minerals.
    -Vegetable oils to provide recommended levels of
    essential fatty acids.
    -Milk-white color, milk-like consistency and pleasant
    aroma.

Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6%
sugar (sucrose), 2.1 % soy oil, 1.9% soy protein isolate,
1.4% coconut oil, 0.15% calcium citrate, 0. 11 % calcium
phosphate tribasic, potassium citrate, potassium
phosphate monobasic, potassium chloride, mono- and
disglycerides, soy lecithin, carrageenan, ascorbic acid,
L-methionine, magnesium chloride, potassium phosphate
dibasic, sodium chloride, choline chloride, taurine,
ferrous sulfate, m-inositol, alpha-tocopheryl acetate,
zinc sulfate, L-carnitine, niacinamide, calcium
pantothenate, cupric sulfate, vitamin A palmitate,
thiamine chloride hydrochloride, riboflavin, pyridoxine
hydrochloride, folic acid, manganese sulfate, potassium
iodide, phylloquinone, biotin, sodium selenite, vitamin
D3 and cyanocobalamin.

B.   Isomil® DF Soy Formula For Diarrhea:

Usage: As a short-term feeding for the dietary management
of diarrhea in infants and toddlers.

Features:
-First infant formula to contain added dietary fiber from
soy fiber specifically for diarrhea management.
-Clinically shown to reduce the duration of loose, watery
stools during mild to severe diarrhea in infants.
-Nutritionally complete to meet the nutritional needs of
the infant.
-Soy protein isolate with added L-methionine meets or
exceeds an infant's requirement for all essential amino
acids.
-Lactose-free formulation to avoid lactose-associated
diarrhea.
-Low osmolality (240 mOsm/kg water) to reduce the risk of
osmotic diarrhea.

-Dual carbohydrates (corn syrup and sucrose) designed to
enhance carbohydrate absorption and reduce the risk of
exceeding the absorptive capacity of the damaged gut.
-Meets or exceeds the vitamin and mineral levels
5   recommended by the Committee on Nutrition of the American
Academy of Pediatrics and required by the Infant Formula
Act.
-1.8 mg of iron (as ferrous sulfate) per 100 Calories to
help prevent iron deficiency.
10   -Vegetable oils to provide recommended levels of
essential fatty acids.

Ingredients: (Pareve) 86% water, 4.8% corn syrup, 2.5%
sugar (sucrose), 2.1% soy oil, 2.0% soy protein isolate,
15   1.4% coconut oil, 0.77% soy fiber, 0.12% calcium citrate,
0.11% calcium phosphate tribasic, 0.10% potassium
citrate, potassium chloride, potassium phosphate
monobasic, mono and diglycerides, soy lecithin,
carrageenan, magnesium chloride, ascorbic acid, L-
20   methionine, potassium phosphate dibasic, sodium chloride,
choline chloride, taurine, ferrous sulfate, m-inositol,
alpha-tocopheryl acetate, zinc sulfate, L-carnitine,
niacinamide, calcium pantothenate, cupric sulfate,
vitamin A palmitate, thiamine chloride hydrochloride,
25   riboflavin, pyridoxine hydrochloride, folic acid,
manganese sulfate, potassium iodide, phylloquinone,
biotin, sodium selenite, vitamin D3 and cyanocobalamin.

C.   Isomil® SF Sucrose-Free Soy Formula With Iron:
30
Usage: As a beverage for infants, children and adults
with an allergy or sensitivity to cow's-milk protein or
an intolerance to sucrose. A feeding for patients with
disorders for which lactose and sucrose should be
35   avoided.

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Features:

-Soy protein isolate to avoid symptoms of cow's-milk-
protein allergy or sensitivity.

-Lactose-free formulation to avoid lactose-associated
diarrhea (carbohydrate source is Polycose® Glucose
Polymers).

-Sucrose free for the patient who cannot tolerate
sucrose.

-Low osmolality (180 mOsm/kg water) to reduce risk of
osmotic diarrhea.

-1.8 mg of iron (as ferrous sulfate) per 100 Calories to
help prevent iron deficiency.

-Recommended levels of vitamins and minerals.

-Vegetable oils to provide recommended levels of
essential fatty acids.

-Milk-white color, milk-like consistency and pleasant
aroma.

Ingredients: (Pareve) 75% water, 11.8% hydrolized
cornstarch, 4.1% soy oil, 4.1 % soy protein isolate, 2.8%
coconut oil, 1.0% modified cornstarch, 0.38% calcium
phosphate tribasic, 0. 17% potassium citrate, 0.13%
potassium chloride, mono- and diglycerides, soy lecithin,
magnesium chloride, abscorbic acid, L-methionine, calcium
carbonate, sodium chloride, choline chloride,
carrageenan, taurine, ferrous sulfate, m-inositol, alpha-
tocopheryl acetate, zinc sulfate,L-carnitine,
niacinamide, calcium pantothenate, cupric sulfate,
vitamin A palmitate, thiamine chloride hydrochloride,
riboflavin, pyridoxine hydrochloride, folic acid,
manganese sulfate, potassium iodide, phylloquinone,
biotin, sodium selenite, vitamin D3 and cyanocobalamin.

D.   Isomil® 20 Soy Formula With Iron Ready To Feed,

20 Cal/fl oz.:
Usage: When a soy feeding is desired.

Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6%
sugar(sucrose), 2.1 % soy oil, 1.9% soy protein isolate,
1.4% coconut oil, 0.15% calcium citrate, 0. 11% calcium
phosphate tribasic, potassium citrate, potassium
phosphate monobasic, potassium chloride, mono- and
diglycerides, soy lecithin, carrageenan, abscorbic acid,
L-methionine, magnesium chloride, potassium phosphate
dibasic, sodium chloride, choline chloride, taurine,
ferrous sulfate, m-inositol, alpha-tocopheryl acetate,
zinc sulfate, L-carnitine, niacinamide, calcium
pantothenate, cupric sulfate, vitamin A palmitate,
thiamine chloride hydrochloride, riboflavin, pyridoxine
hydrochloride, folic
acid, manganese sulfate, potassium iodide, phylloquinone,
biotin, sodium selenite, vitamin D3 and cyanocobalamin.

E.   Similac® Infant Formula:

Usage: When an infant formula is needed: if the decision
is made to discontinue breastfeeding before age 1 year,
if a supplement to breastfeeding is needed or as a
routine feeding if breastfeeding is not adopted.

Features:
-Protein of appropriate quality and quantity for good
growth; heat-denatúred, which reduces the risk of milk-
associated enteric blood loss.
-Fat from a blend of vegetable oils (doubly homogenized),
providing essential linoleic acid that is easily
absorbed.
-Carbohydrate as lactose in proportion similar to that of
human milk.

-Low renal solute load to minimize stress on developing
organs.
-Powder, Concentrated Liquid and Ready To Feed forms.
Ingredients: (-D) Water, nonfat milk, lactose, soy oil,

5   coconut oil, mono- and diglycerides, soy lecithin,
abscorbic acid, carrageenan, choline chloride, taurine,
m-inositol, alpha-tocopheryl acetate, zinc sulfate,
niacinamide, ferrous sulfate, calcium pantothenate,
cupric sulfate, vitamin A palmitate, thiamine chloride

10   hydrochloride, riboflavin, pyridoxine hydrochloride,
folic
acid, manganese sulfate, phylloquinone, biotin, sodium
selenite, vitamin D3 and cyanocobalamin.

15   F.   Similac® NeoCare Premature Infant Formula With Iron:

Usage: For premature infants' special nutritional needs
after hospital discharge. Similac NeoCare is a
nutritionally complete formula developed to

20   provide premature infants with extra calories, protein,
vitamins and minerals needed to promote catch-up growth
and support development.

Features:

25   -Reduces the need for caloric and vitamin
supplementation. More calories (22 Cal/fl oz) than
standard term formulas (20 Cal/fl oz).
-Highly absorbed fat blend, with medium-chain
triglycerides

30   (MCToil) to help meet the special digestive needs of
premature infants.
-Higher levels of protein, vitamins and minerals per 100
calories to extend the nutritional support initiated in-
hospital.

-More calcium and phosphorus for improved bone
mineralization.

Ingredients: -D Corn syrup solids, nonfat milk, lactose,
whey protein concentrate, soy oil, high-oleic safflower
oil, fractionated coconut oil (medium chain
triglycerides), coconut oil, potassium citrate, calcium
phosphate tribasic, calcium carbonate, ascorbic acid,
magnesium chloride, potassium chloride, sodium chloride,
taurine, ferrous sulfate, m-inositol, choline chloride,
ascorbyl palmitate, L-carnitine, alpha-tocopheryl
acetate, zinc sulfate, niacinamide, mixed tocopherols,
sodium citrate, calcium pantothenate, cupric sulfate,
thiamine chloride hydrochloride, vitamin A palmitate,
beta carotene, riboflavin, pyridoxine hydrochloride,
folic acid, manganese sulfate, phylloquinone, biotin,
sodium selenite, vitamin D3 and cyanocobalamin.

G.   Similac Natural Care Low-Iron Human Milk Fortifier
Ready To Use, 24 Cal/fl oz.:

Usage: Designed to be mixed with human milk or to be fed
alternatively with human milk to low-birth-weight
infants.

Ingredients: -D Water, nonfat milk, hydrolyzed
cornstarch, lactose, fractionated coconut oil (medium-
chain triglycerides), whey protein concentrate, soy oil,
coconut oil, calcium phosphate tribasic, potassium
citrate, magnesium chloride, sodium citrate, ascorbic
acid, calcium carbonate, mono and diglycerides, soy
lecithin, carrageenan, choline chloride, m-inositol,
taurine, niacinamide, L-carnitine, alpha tocopheryl
acetate, zinc sulfate, potassium chloride, calcium
pantothenate, ferrous sulfate, cupric sulfate,

riboflavin, vitamin A palmitate, thiamine chloride
hydrochloride, pyridoxine hydrochloride, biotin, folic
acid, manganese sulfate, phylloquinone, vitamin D3,
sodium selenite and cyanocobalamin.

5   Various PUFAs of this invention can be substituted
and/or added to the infant formulae described above and
to other infant formulae known to those in the art.

II. NUTRITIONAL FORMULATIONS
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A. ENSURE®
Usage: ENSURE is a low-residue liquid food designed
primarily as an oral nutritional supplement to be used
with or between meals or, in appropriate amounts, as a
15   meal replacement. ENSURE is lactose- and gluten-free, and
is suitable for use in modified diets, including low-
cholesterol diets. Although it is primarily an oral
supplement, it can be fed by tube.

20   Patient Conditions:
-For patients on modified diets
-For elderly patients at nutrition risk
-For patients with involuntary weight loss
-For patients recovering from illness or surgery
25   -For patients who need a low-residue diet

Ingredients: -D Water, Sugar (Sucrose), Maltodextrin
(Corn), Calcium and Sodium Caseinates, High-Oleic
Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil,
30   Potassium Citrate, Calcium Phosphate Tribasic, Sodium
Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic,
Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline
Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate,
Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum,
35   Niacinamide,

Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate,
Vitamin A Palmitate, Thiamine Chloride Hydrochloride,
Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Sodium
Molybdate, Chromium Chloride, Biotin, Potassium Iodide,
5   Sodium Selenate.

B.ENSURE® BARS:

Usage: ENSURE BARS are complete, balanced nutrition for
10   supplemental use between or with meals. They provide a
delicious, nutrient-rich alternative to other snacks.
ENSURE BARS contain <1 g lactose/bar, and Chocolate Fudge
Brownie flavor is gluten-free. (Honey Graham Crunch
flavor contains gluten.)
15

Patient Conditions:
-For patients who need extra calories, protein, vitamins
and minerals.
-Especially useful for people who do not take in enough
20   calories and nutrients.
-For people who have the ability to chew and swallow
-Not to be used by anyone with a peanut allergy or any
type of allergy to nuts.
Ingredients: Honey Graham Crunch -- High-Fructose Corn
25   Syrup, Soy Protein Isolate, Brown Sugar, Honey,
Maltodextrin (Corn), Crisp Rice (Milled Rice,
Sugar [Sucrose], Salt [Sodium Chloride] and Malt), Oat
Bran, Partially Hydrogenated Cottonseed and Soy Oils, Soy
Polysaccharide, Glycerine, Whey Protein Concentrate,
30   Polydextrose, Fructose, Calcium Caseinate, Cocoa Powder,
Artificial Flavors, Canola Oil, High-Oleic Safflower Oil,
Nonfat Dry Milk, Whey Powder, Soy Lecithin and Corn Oil.
Manufactured in a facility that processes nuts.

Vitamins and Minerals: Calcium Phosphate Tribasic,
Potassium Phosphate Dibasic, Magnesium Oxide, Salt
(Sodium Chloride), Potassium Chloride, Ascorbic Acid,
Ferric Orthophosphate, Alpha-Tocopheryl Acetate,
Niacinamide, Zinc Oxide, Calcium Pantothenate, Copper
Gluconate, Manganese Sulfate, Riboflavin, Beta Carotene,
Pyridoxine Hydrochloride, Thiamine Mononitrate, Folic
Acid, Biotin,
Chromium Chloride, Potassium Iodide, Sodium Selenate,
Sodium Molybdate, Phylloquinone, Vitamin D3 and
Cyanocobalamin.

Protein: Honey Graham Crunch - The protein source is a
blend of soy protein isolate and milk proteins.

Soy protein isolate	74%
Milk proteins	26%

Fat: Honey Graham Crunch - The fat source is a blend of
partially hydrogenated cottonseed and soybean, canola,
high oleic safflower, oils, and soy lecithin.

Partially hydrogenated cottonseed and soybean oil 76%

Canola oil	8%
High-oleic safflower oil	8%
Corn oil	4%
Soy lecithin	4%

Carbohydrate: Honey Graham Crunch - The carbohydrate
source is a combination of high-fructose corn syrup,
brown sugar, maltodextrin, honey, crisp rice, glycerine,
soy polysaccharide, and oat bran.

High-fructose corn syrup	24%
Brown sugar	21%
Maltodextrin	12%

Honey	11%
Crisp rice	9%
Glycerine	9%
Soy Polysaccharide	7%
Oat bran	7%

C.   ENSURE® HIGH PROTEIN:

Usage: ENSURE HIGH PROTEIN is a concentrated, high-protein liquid food designed for people who require additional calories, protein, vitamins, and minerals in their diets. It can be used as an oral nutritional supplement with or between meals or, in appropriate amounts, as a meal replacement. ENSURE HIGH PROTEIN is lactose- and gluten-free, and is suitable for use by people recovering from general surgery or hip fractures and by patients at risk for pressure ulcers.

Patient Conditions:
-For patients who require additional calories, protein, vitamins, and minerals, such as patients recovering from general surgery or hip fractures, patients at risk for pressure ulcers, and patients on low-cholesterol diets.

Features:
-Low in saturated fat
-Contains 6 g of total fat and < 5 mg of cholesterol per serving
-Rich, creamy taste
-Excellent source of protein, calcium, and other essential vitamins and minerals
-For low-cholesterol diets
-Lactose-free, easily digested

Ingredients:

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Vanilla Supreme: -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-OIeic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Suffate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide,
Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:
  The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates	85%
Soy protein isolate	15%

Fat:
  The fat source is a blend of three oils: high-oleic safflower, canola, and soy.

High-oleic safflower oil	40%
Canola oil	30%
Soy oil	30%

The level of fat in ENSURE HIGH PROTEIN meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE HIGH PROTEIN represent 24% of the total calories, with 2.6% of the fat being from saturated fatty acids and

7.9% from polyunsaturated fatty acids. These values are within the AHA guidelines of < 30% of total calories from fat, < 10% of the calories from saturated fatty acids, and < 10% of total calories from polyunsaturated fatty acids.

Carbohydrate:

ENSURE HIGH PROTEIN contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla supreme, chocolate royal, wild berry, and banana), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.
Vanilla and other nonchocolate flavors:

Sucrose   60%
Maltodextrin   40%

Chocolate:

Sucrose   70%
Maltodextrin   30%

D. ENSURE® LIGHT
Usage: ENSURE LIGHT is a low-fat liquid food designed for use as an oral nutritional supplement with or between meals. ENSURE LIGHT is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions:

-For normal-weight or overweight patients who need extra nutrition in a supplement that contains 50% less fat and 20% fewer calories than ENSURE.

-For healthy adults who do not eat right and need extra
nutrition.
Features:

5   -Low in fat and saturated fat
-Contains 3 g of total fat per serving and < 5 mg
cholesterol
-Rich, creamy taste
-Excellent source of calcium and other essential vitamins
10   and minerals
-For low-cholesterol diets
-Lactose-free, easily digested

Ingredients:

15

French Vanilla: -D Water, Maltodextrin (Corn), Sugar
(Sucrose), Calcium Caseinate, High-Oleic Safflower Oil,
Canola Oil, Magnesium Chloride, Sodium Citrate, Potassium
Citrate, Potassium Phosphate Dibasic, Magnesium Phosphate
20   Dibasic, Natural and Artificial Flavor, Calcium Phosphate
Tribasic, Cellulose Gel, Choline Chloride, Soy Lecithin,
Carrageenan, Salt (Sodium Chloride), Ascorbic Acid,
Cellulose Gum, Ferrous Sulfate, Alpha-Tocopheryl Acetate,
Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium
25   Pantothenate, Cupric Sulfate, Thiamine Chloride
Hydrochloride, Vitamin A Palmitate, Pyridoxine
Hydrochloride, Riboflavin, Chromium Chloride, Folic Acid,
Sodium Molybdate, Biotin, Potassium Iodide, Sodium
Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

30

Protein:
The protein source is calcium caseinate.

Calcium caseinate   100%

35

Fat:

The fat source is a blend of two oils: high-oleic
safflower and canola.

High-oleic safflower oil   70%
Canola oil   30%

The level of fat in ENSURE LIGHT meets American Heart
Association (AHA) guidelines. The 3 grams of fat in
ENSURE LIGHT represent 13.5% of the total calories, with
1.4% of the fat being from saturated fatty acids and 2.6%
from polyunsaturated fatty acids. These values are
within the AHA guidelines of < 30% of total calories from
fat, < 10% of the, calories from saturated fatty acids,
and < 10% of total calories from polyunsaturated fatty
acids.

Carbohydrate:
ENSURE LIGHT contains a combination of maltodextrin and
sucrose. The chocolate flavor contains corn syrup as
well. The mild sweetness and flavor variety (French
vanilla, chocolate supreme, strawberry swirl), plus VARI-
FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon,
and
orange, help to prevent flavor fatigue and aid in patient
compliance.

Vanilla and other nonchocolate flavors:

Sucrose   51%
Maltodextrin   49%

Chocolate:

Sucrose	47.0%
Corn Syrup	26.5%
Maltodextrin	26.5%

Vitamins and Minerals:

An 8-fl-oz serving of ENSURE LIGHT provides at least 25% of the RDIs for 24 key vitamins and minerals.

Caffeine:

Chocolate flavor contains 2.1 mg caffeine/8 fl oz.

E.ENSURE PLUS®

Usage: ENSURE PLUS is a high-calorie, low-residue liquid food for use when extra calories and nutrients, but a normal concentration of protein, are needed. It is designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement. ENSURE PLUS is lactose- and gluten-free. Although it is primarily an oral nutritional supplement, it can be fed by tube.

Patient Conditions:

-For patients who require extra calories and nutrients, but a normal concentration of protein, in a limited volume.

-For patients who need to gain or maintain healthy weight.

Features:

-Rich, creamy taste

-Good source of essential vitamins and minerals

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Ingredients:

Vanilla: -D Water, Corn Syrup, Maltodextrin (Corn), Corn
Oil, Sodium and Calcium Caseinates, Sugar (Sucrose), Soy
5   Protein Isolate, Magnesium Chloride, Potassium Citrate,
Calcium Phosphate Tribasic, Soy Lecithin, Natural and
Artificial Flavor, Sodium Citrate, Potassium Chloride,
Choline Chloride, Ascorbic Acid, Carrageenan, Zinc
Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate,
10   Niacinamide, Calcium Pantothenate, Manganese Sulfate,
Cupric
Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine
Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic
Acid, Biotin, Chromium Chloride, Sodium Molybdate,
15   Potassium Iodide, Sodium Selenite, Phylloquinone,
Cyanocobalamin and Vitamin D3.

Protein:
       The protein source is a blend of two high-biologic-
20   value proteins: casein and soy.

Sodium and calcium caseinates   84%
Soy protein isolate   16%
Fat:
25   The fat source is corn oil.

Corn oil   100%

Carbohydrate:
30   ENSURE PLUS contains a combination of maltodextrin
and sucrose. The mild sweetness and flavor variety
(vanilla, chocolate, strawberry, coffee, buffer pecan,
and eggnog), plus VARI-

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FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla, strawberry, butter pecan, and coffee flavors:

Corn Syrup	39%
Maltodextrin	38%
Sucrose	23%

Chocolate and eggnog flavors:

Corn Syrup	36%
Maltodextrin	34%
Sucrose	30%

Vitamins and Minerals:
An 8-fl-oz serving of ENSURE PLUS provides at least 15% of the RDIs for 25 key Vitamins and minerals.
Caffeine:
Chocolate flavor contains 3.1 mg Caffeine/8 fl oz. Coffee flavor contains a trace amount of caffeine.

F. ENSURE PLUS® HN

Usage: ENSURE PLUS HN is a nutritionally complete high-calorie, high-nitrogen liquid food designed for people with higher calorie and protein needs or limited volume tolerance. It may be used for oral supplementation or for total nutritional support by tube. ENSURE PLUS HN is lactose- and gluten-free.

Patient Conditions:
-For patients with increased calorie and protein needs, such as following surgery or injury.

-For patients with limited volume tolerance and early satiety.

Features:

-For supplemental or total nutrition

-For oral or tube feeding

-1.5 CaVmL,

-High nitrogen

-Calorically dense

Ingredients:

Vanilla: -D Water, Maltodextrin (Corn), Sodium and Calcium Caseinates, Corn Oil, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor, Sodium Citrate, Choline Chloride, Ascorbic Acid, Taurine, L-Carnitine, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Carrageenan, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D3.

G. ENSURE® POWDER:

Usage: ENSURE POWDER (reconstituted with water) is a low-residue liquid food designed primarily as an oral nutritional supplement to be used with or between meals. ENSURE POWDER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions:

-For patients on modified diets

-For elderly patients at nutrition risk

-For patients recovering from illness/surgery

-For patients who need a low-residue diet

Features:

-Convenient, easy to mix

-Low in saturated fat

-Contains 9 g of total fat and < 5 mg of cholesterol per serving

-High in vitamins and minerals

-For low-cholesterol diets

-Lactose-free, easily digested

Ingredients: -D Corn Syrup, Maltodextrin (Corn), Sugar (Sucrose), Corn Oil, Sodium and Calcium Caseinates, Soy Protein Isolate, Artificial Flavor, Potassium Citrate, Magnesium Chloride, Sodium Citrate, Calcium Phosphate Tribasic, Potassium Chloride, Soy Lecithin, Ascorbic Acid, Choline Chloride, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Thiamine Chloride Hydrochloride, Cupric Sulfate, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Sodium Molybdate, Chromium Chloride, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates	84%
Soy protein isolate	16%

Fat:

   The fat source is corn oil.

Corn oil   100%

Carbohydrate:

   ENSURE POWDER contains a combination of corn syrup, maltodextrin, and sucrose. The mild sweetness of ENSURE POWDER, plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, helps to prevent flavor fatigue and aid in patient compliance.

Vanilla:

Corn Syrup   35%
Maltodextrin   35%
Sucrose   30%

H.   ENSURE® PUDDING

Usage: ENSURE PUDDING is a nutrient-dense supplement providing balanced nutrition in a nonliquid form to be used with or between meals. It is appropriate for consistency-modified diets (e.g., soft, pureed, or full liquid) or for people with swallowing impairments. ENSURE PUDDING is gluten-free.
Patient Conditions:
-For patients on consistency-modified diets (e.g., soft, pureed, or full liquid)
-For patients with swallowing impairments

Features:
-Rich and creamy, good taste
-Good source of essential vitamins and minerals
-Convenient-needs no refrigeration

-Gluten-free

Nutrient Profile per 5 oz: Calories 250, Protein 10.9%,
Total Fat 34.9%, Carbohydrate 54.2%

Ingredients:

Vanilla: -D Nonfat Milk, Water, Sugar (Sucrose),
Partially Hydrogenated Soybean Oil, Modified Food Starch,
Magnesium Sulfate, Sodium Stearoyl Lactylate, Sodium
Phosphate Dibasic, Artificial Flavor, Ascorbic Acid, Zinc
Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate,
Choline Chloride, Niacinamide, Manganese Sulfate, Calcium
Pantothenate, FD&C Yellow #5, Potassium Citrate, Cupric
Sulfate, Vitamin A Palmitate, Thiamine Chloride
Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, FD&C
Yellow #6, Folic Acid, Biotin, Phylloquinone, Vitamin D3
and Cyanocobalamin.

Protein:

    The protein source is nonfat milk.

Nonfat milk   100%
Fat:
    The fat source is hydrogenated soybean oil.

Hydrogenated soybean oil   100%

Carbohydrate:

    ENSURE PUDDING contains a combination of sucrose and
modified food starch. The mild sweetness and flavor
variety (vanilla, chocolate, butterscotch, and tapioca)
help prevent flavor fatigue. The product contains 9.2
grams of lactose per serving.

Vanilla and other nonchocolate flavors:

Sucrose	56%
Lactose	27%
Modified food starch	17%

Chocolate:

Sucrose	58%
Lactose	26%
Modified food starch	16%

I.   ENSURE® WITH FIBER:

Usage: ENSURE WITH FIBER is a fiber-containing,
nutritionally complete liquid food designed for people
who can benefit from increased dietary fiber and
nutrients. ENSURE WITH FIBER is suitable for people who
do not require a low-residue diet. It can be fed orally
or by tube, and can be used as a
nutritional supplement to a regular diet or, in
appropriate amounts, as a meal replacement. ENSURE WITH
FIBER is lactose- and gluten-free, and is suitable for
use in modified diets, including low-cholesterol diets.

Patient Conditions:
-For patients who can benefit from increased dietary
fiber and nutrients

Features:
-New advanced formula-low in saturated fat, higher in
vitamins and minerals
-Contains 6 g of total fat and < 5 mg of cholesterol per
serving
-Rich, creamy taste

-Good source of fiber

-Excellent source of essential vitamins and minerals

-For low-cholesterol diets

-Lactose- and gluten-free

Ingredients:

Vanilla: -D Water; Maltodextrin (Corn), Sugar (Sucrose),
Sodium and Calcium Caseinates, Oat Fiber, High-Oleic
Safflower Oil, Canola Oil, Soy Protein Isolate, Corn Oil,
Soy Fiber, Calcium Phosphate Tribasic, Magnesium
Chloride, Potassium Citrate, Cellulose Gel, Soy Lecithin,
Potassium Phosphate Dibasic, Sodium Citrate, Natural and
Artificial Flavors, Choline Chloride, Magnesium
Phosphate, Ascorbic Acid, Cellulose Gum, Potassium
Chloride, Carrageenan, Ferrous Sulfate, Alpha-Tocopheryl
Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate,
Calcium Pantothenate, Cupric Sulfate, Vitamin A
Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine
Hydrochloride, Riboflavin, Folic Acid, Chromium Chloride,
Biotin, Sodium Molybdate, Potassium Iodide, Sodium
Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is a blend of two high-biologic-
value proteins-casein and soy.

Sodium and calcium caseinates   80%
Soy protein isolate   20%

Fat:

The fat source is a blend of three oils: high-oleic
safflower, canola, and corn.

High-oleic safflower oil   40%

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Canola oil	40%
Corn oil	20%

The level of fat in ENSURE WITH FIBER meets American
Heart Association (AHA) guidelines. The 6 grams of fat in
ENSURE WITH FIBER represent 22% of the total calories,
with 2.01 % of the fat being from saturated fatty acids
and 6.7% from polyunsaturated fatty acids. These values
are within the AHA guidelines of $\leq$ 30% of total calories
from fat, < 10% of the calories from saturated fatty
acids, and $\leq$ 10% of total calories from polyunsaturated
fatty acids.

Carbohydrate:

ENSURE WITH FIBER contains a combination of
maltodextrin and sucrose. The mild sweetness and flavor
variety (vanilla, chocolate, and butter pecan), plus
VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry,
lemon, and orange, help to prevent flavor fatigue and aid
in patient compliance.

Vanilla and other nonchocolate flavors:

Maltodextrin	66%
Sucrose	25%
Oat Fiber	7%
Soy Fiber	2%

Chocolate:

Maltodextrin	55%
Sucrose	36%
Oat Fiber	7%
Soy Fiber	2%

Fiber:

The fiber blend used in ENSURE WITH FIBER consists of oat fiber and soy polysaccharide. This blend results in approximately 4 grams of total dietary fiber per 8-fl. oz can. The ratio of insoluble to soluble fiber is 95:5.

The various nutritional supplements described above and known to others of skill in the art can be substituted and/or supplemented with the PUFAs produced in accordance with the present invention.

J.Oxepa™ Nutritional Product

Oxepa is a low-carbohydrate, calorically dense, enteral nutritional product designed for the dietary management of patients with or at risk for ARDS. It has a unique combination of ingredients, including a patented oil blend containing eicosapentaenoic acid (EPA from fish oil), γ-linolenic acid (GLA from borage oil), and elevated antioxidant levels.

Caloric Distribution:

Caloric density is high at 1.5 Cal/mL (355 Cal/8 fl oz), to minimize the volume required to meet energy needs. The distribution of Calories in Oxepa is shown in Table A.

Table A. Caloric Distribution of Oxepa

	per 8 fl oz.	per liter	% of Cal
Calories	355	1,500	---
Fat (g)	22.2	93.7	55.2
Carbohydrate (g)		25	105.528.1
Protein (g)	14.8	62.5	16.7
Water (g)	186	785	---

Fat:

-Oxepa contains 22.2 g of fat per 8-fl oz serving (93.7 g/L).

-The fat source is an oil blend of 31.8% canola oil, 25% medium-chain triglycerides (MCTs), 20% borage oil, 20% fish oil, and 3.2 % soy lecithin. The typical fatty acid profile of Oxepa is shown in Table B.

-Oxepa provides a balanced amount of polyunsaturated, monounsaturated, and saturated fatty acids, as shown in Table VI.

-Medium-chain trigylcerides (MCTs) -- 25% of the fat blend -- aid gastric emptying because they are absorbed by the intestinal tract without emulsification by bile acids.

The various fatty acid components of Oxepa™ nutritional product can be substituted and/or supplemented with the PUFAs produced in accordance with this invention.

Table B. Typical Fatty Acid Profile

	% Total Fatty Acids	g/8 fl oz*	9/L*
Caproic (6:0)	0.2	0.04	0.18
Caprylic (8:0)	14.69	3.1	13.07
Capric (10:0)	11.06	2.33	9.87
Palmitic (16:0)	5.59	1.18	4.98
Palmitoleic	1.82	0.38	1.62
Stearic	1.94	0.39	1.64
Oleic	24.44	5.16	21.75
Linoleic	16.28	3.44	14.49
α-Linolenic	3.47	0.73	3.09
γ-Linolenic	4.82	1.02	4.29
Eicosapentaenoic	5.11	1.08	4.55

n-3-Docosapent- aenoic	0.55	0.12	0.49
Docosahexaenoic	2.27	0.48	2.02
Others	7.55	1.52	6.72

Fatty acids equal approximately 95% of total fat.

Table C. Fat Profile of Oxepa.

% of total calories from fat	55.2
Polyunsaturated fatty acids	31.44 g/L
Monounsaturated fatty acids	25.53 g/L
Saturated fatty acids	32.38 g/L
n-6 to n-3 ratio	1.75:1
Cholesterol	9.49 mg/8 fl oz
	40.1 mg/L

Carbohydrate:

-The carbohydrate content is 25.0 g per 8-fl-oz serving (105.5 g/L).

-The carbohydrate sources are 45% maltodextrin (a complex carbohydrate) and 55% sucrose (a simple sugar), both of which are readily digested and absorbed.

-The high-fat and low-carbohydrate content of Oxepa is designed to minimize carbon dioxide (CO_2) production. High CO_2 levels can complicate weaning in ventilator-dependent patients. The low level of carbohydrate also may be useful for those patients who have developed stress-induced hyperglycemia.

-Oxepa is lactose-free.

Dietary carbohydrate, the amino acids from protein, and the glycerol moiety of fats can be converted to glucose within the body. Throughout this process, the carbohydrate requirements of glucose-dependent tissues (such as the central nervous system and red blood cells) are met. However, a diet free of carbohydrates can lead

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to ketosis, excessive catabolism of tissue protein, and
loss of fluid and electrolytes. These effects can be
prevented by daily ingestion of 50 to 100 g of digestible
carbohydrate, if caloric intake is adequate. The
carbohydrate level in Oxepa is also sufficient to
minimize gluconeogenesis, if energy needs are being met.

Protein:
-Oxepa contains 14.8 g of protein per 8-fl-oz serving
(62.5 g/L).
-The total calorie/nitrogen ratio (150:1) meets the need
of stressed patients.
-Oxepa provides enough protein to promote anabolism and
the maintenance of lean body mass without precipitating
respiratory problems. High protein intakes are a concern
in patients with respiratory insufficiency. Although
protein has little effect on CO_2 production, a high
protein diet will increase ventilatory drive.
-The protein sources of Oxepa are 86.8% sodium caseinate
and 13.2% calcium caseinate.
- The amino acid profile of the protein system in Oxepa
meets or surpasses the standard for high quality protein
set by the National Academy of Sciences.

* Oxepa is gluten-free.

CLAIMS:

1.   An isolated nucleotide sequence or fragment thereof
comprising or complementary to a nucleotide sequence
encoding a polypeptide having desaturase activity,
wherein the amino acid sequence of said polypeptide has
at least 50% identity to an amino acid sequence selected
from the group consisting of SEQ ID NO:18, SEQ ID NO:19,
SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID NO:46
and SEQ ID NO:55.

2.   An isolated nucleotide sequence or fragment thereof
comprising or complementary to a nucleotide sequence
having at least 50% identity to a nucleotide sequence
selected from the group consisting of SEQ ID NO:14, SEQ
ID NO:15 and SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:36,
SEQ ID NO:45 and SEQ ID NO:54.

3.   The isolated nucleotide sequence of claim 1 or 2
wherein said sequence encodes a functionally active
desaturase which utilizes a monounsaturated or
polyunsaturated fatty acid as a substrate.

4.   The nucleotide sequence of claim 1 or 2 wherein said
sequence is derived from an organism selected from the
group consisting of a fungus and an algae.

5.   The nucleotide sequence of claim 4 wherein said
sequence selected from the group consisting of SEQ ID
NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17 and SEQ
ID NO:45 is derived from the fungus *Thraustochytrium
aureum*, said sequence comprising SEQ ID NO:36 is derived
from the fungus *Schizochytrium aggregatum*, and said
sequence comprising SEQ ID NO:54 is derived from the
algae *Isochrysis galbana*.

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6.   A purified polypeptide encoded by said nucleotide
    sequence of claims 1 or 2.

7.   A purified polypeptide which desaturates
polyunsaturated fatty acids, at carbon 4 and has an amino
acid sequence having at least 50% identity to an amino
acid sequence selected from the group consisting of SEQ
ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ
ID NO:37, SEQ ID NO:46 and SEQ ID NO:55.

8.   A method of producing a desaturase comprising the
steps of:

    a) isolating a nucleotide sequence comprising or
        complementary to a nucleotide sequence:

        i) encoding a polypeptide having an amino acid
        sequence having at least 50% identity to an
        amino acid sequence selected from the group
        consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ
        ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID
        NO:46 and SEQ ID NO:55 or
        ii) having at least 50% identity to a
        nucleotide sequence selected from the group
        consisting of SEQ ID NO:14, SEQ ID NO:15, SEQ
        ID NO:16, SEQ ID NO:17, SEQ ID NO:36, SEQ ID
        NO:45 and SEQ ID NO:54;

    b) constructing a vector comprising: i) said isolated
        nucleotide sequence operably linked to ii) a
        promoter;

    c) introducing said vector into a host cell for a

time and under conditions sufficient for
expression of said desaturase.

9.   A vector comprising:

      a) an isolated nucleotide sequence comprising or
complementary to a nucleotide sequence:
            i) encoding a polypeptide having an amino acid
            sequence having at least 50% identity to an
            amino acid sequence selected from the group
            consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ
            ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID
            NO:46 and SEQ ID NO:55 or
            ii) having at least 50% identity to a
            nucleotide sequence selected from the group
            consisting of SEQ ID NO:14, SEQ ID NO:15, SEQ
            ID NO:16, SEQ ID NO:17, SEQ ID NO:36, SEQ ID
            NO:45 and SEQ ID NO:54, operably linked to
      b) a promoter.

10.   A host cell comprising said vector of claim 9.

11.   A plant cell, plant or plant tissue comprising
said vector of claim 9, wherein expression of said
nucleotide sequence of said vector results in production
of a polyunsaturated fatty acid by said plant cell, plant
or plant tissue.

12.   The plant cell, plant or plant tissue of claim 11
wherein said polyunsaturated fatty acid is selected from
the group consisting of ω6-docosapentaenoic acid and
docosahexaenoic acid.

13.   One or more plant oils or acids expressed by said
plant cell, plant or plant tissue of claim 11.

14. A transgenic plant comprising said vector of claim 9, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid in seeds of said transgenic plant.

15. A method for producing a polyunsaturated fatty acid comprising the steps of:

a)   isolating a nucleotide sequence comprising or complementary to a nucleotide sequence:

    i)   encoding a polypeptide having an amino acid sequence having at least 50% identity to an amino acid sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:37, SEQ ID NO:46 and SEQ ID NO:55 or

    ii)   having at least 50% identity to a nucleotide sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16 and SEQ ID NO:17, SEQ ID NO:36, SEQ ID NO:45, and SEQ ID NO:54;

b)   constructing a vector comprising said isolated nucleotide sequence;

c)   introducing said vector into a host cell for a time and under conditions sufficient for expression of $\Delta 4$-desaturase; and

d)   exposing said expressed $\Delta 4$-desaturase to a substrate polyunsaturated fatty acid in order to convert said substrate to a product polyunsaturated fatty acid.

16. The method according to claim 15, wherein said substrate polyunsaturated fatty acid is adrenic acid or ω3-docosapentaenoic acid and said product polyunsaturated fatty acid is ω6-docosapentaenoic acid or docosahexaenoic acid, respectively.

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17. The method according to claim 15 further
comprising the step of exposing said product
polyunsaturated fatty acid to a desaturase in order to
· 5   convert said product polyunsaturated fatty acid to
another polyunsaturated fatty acid.

18. The method according to claim 17 wherein said
product polyunsaturated fatty acid is ω6-docosapentaenoic
10   acid and said another polyunsaturated fatty acid is
docosahexaenoic acid.

19. A method of producing a polyunsaturated fatty
acid comprising the steps of:
15   a)   exposing a substrate polyunsaturated fatty
acid to one or more enzymes selected from the group
consisting of a desaturase and an elongase in order to
convert said substrate to a product polyunsaturated fatty
acid; and
20   b)   exposing said product polyunsaturated fatty acid
of step (a) to a Δ4-desaturase comprising an amino acid
sequence selected from the group consisting of SEQ ID
NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID
NO:37, SEQ ID NO:46 and SEQ ID NO:55, in order to convert
25   said product polyunsaturated fatty acid to a final
product polyunsaturated fatty acid.

20. The method of claim 19 wherein said substrate
polyunsaturated fatty acid is selected from the group
30   consisting of linoleic acid, γ-linolenic acid,
stearidonic acid, arachidonic acid, dihomo-γ-linolenic
acid, eicosatetraenoic acid, adrenic acid,
eicosapentaenoic acid.

21. The method of claim 19 wherein said final product polyunsaturated fatty acid is selected from the group consisting of ω6-docosapentaenoic acid and docosahexaenoic acid.

22. A composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of 15 and said another polyunsaturated fatty acid produced according to the method of claim 17.

23. The composition of claim 22 wherein said product polyunsaturated fatty acid is at least one polyunsaturated fatty acid selected from the group consisting of ω6-docosapentaenoic acid and docosahexaenoic acid.

24. The composition of claim 22 wherein said another polyunsaturated fatty acid is docosahexaenoic acid.

25. The composition of claim 22 wherein said composition is selected from the group consisting of an infant formula, a dietary supplement and a dietary substitute.

26. The composition of claim 22 wherein said composition is administered to a human or an animal.

27. The composition of claim 26 wherein said composition is administered enterally or parenterally.

28. A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to said patient said composition

of claim 22 in an amount sufficient to effect said

prevention or treatment.

29.   An isolated nucleotide sequence or fragment

thereof comprising or complementary to a nucleotide

sequence encoding a polypeptide having desaturase

activity, wherein the amino acid sequence of said

polypeptide has at least 30% identity to the amino acid

sequence of SEQ ID NO:55.

30.   An isolated nucleotide sequence or fragment thereof

comprising or complementary to a nucleotide sequence

having at least 40% identity to the nucleotide sequence

of SEQ ID NO:54.

31.   A purified polypeptide which desaturates

polyunsaturated fatty acids at carbon 4 and has an amino

acid sequence having at least 30% identity to the amino

acid sequence of SEQ ID NO:55.
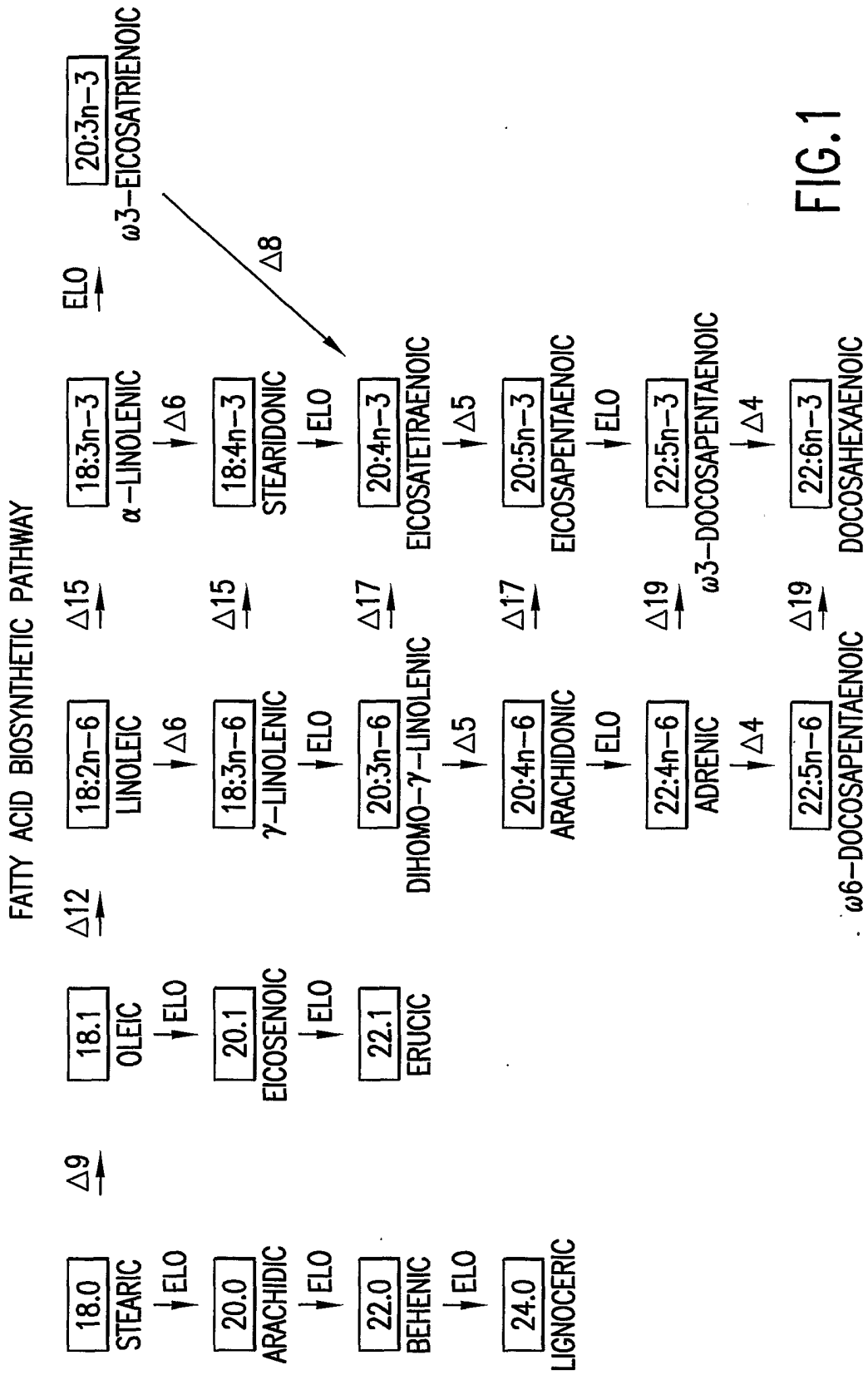
FIG.1

Amino Acid Comparison of Delta-4 Desaturases

```
        1                                                         50
prta6   MTVGFDETVT MDTVRNHNMP DDAWCAIHGT VYDITKFSKV HPGGDIIMLA
prta8   MTVGFDETVT MDTVRNHNMP DDAWCAIHGT VYDITKFSKV HPGGDIIMLA
prta7   MTVGFDETVT MDTVRNHNMP DDAWCAIHGT VYDITKFSKV HPGGDIIMLA
prta5   MTVGFDETVT MDTVRNHNMP DDAWCAIHGT VYDITKFSKV HPGGDIIMLA


        51                                                        100
prta6   AGKEATILFE TYHIKGVPDA VLRKYKVGKL PQGKKGETSH MPTGLDSAFY
prta8   AGKEATILFE TYHIKGVPDA VLRKYKVGKL PQGKKGETSH MPTGLDSASY
prta7   AGKEATILFE TYHIKGVPDA VLRKYKVGKL PQGKKGETSH MPTGLDSASY
prta5   AGKEATILFE TYHIKGVPDA VLRKYKVGKL PQGKKGETSH MPTGLDSASY


        101                                                       150
prta6   YSWDSEFYRV LRERVAKKLA EPGLMQRARM ELWAKAIFLL AGFWGSLYAM
prta8   YSWDSEFYRV LRERVAKKLA EPGLMQRARM ELWAKAIFLL AGFWGSLYAM
prta7   YSWDSEFYRV LRERVAKKLA EPGLMQRARM ELWAKAIFLL AGFWGSLYAM
prta5   YSWDSEFYRV LRERVAKKLA EPGLMQRARM ELWAKAIFLL AGFWGSLYAM


        151                                                       200
prta6   CVLDPHGGAM VAAVTLGVFA AFVGTCIQHD GSHGAFSKSR FMNKAAGWTL
prta8   CVLDPHGGAM VAAVTLGVFA AFVGTCIQHD GSHGAFSKSR FMNKAAGWTL
prta7   CVLDPHGGAM VAAVTLGVFA AFVGTCIQHD GSHGAFSKSR FMNKAAGWTL
prta5   CVLDPHGGAM VAAVTLGVFA AFVGTCIQHD GSHGAFSKSR FMNKAAGWTL


        201                                                       250
prta6   DMIGASAMTW EMQHVLGHHP YTNLIEMENG LAKVKGADVD PKKVDQESDP
prta8   DMIGASAMTW EMQHVLGHHP YTNLIEMENG LAKVKGADVD PKKVDQESDP
prta7   DMIGASAMTW EMQHVLGHHP YTNLIEMENG LAKVKGADVD PKKVDQESDP
prta5   DMIGASAMTW EMQHVLGHHP YTNLIEMENG LAKVKGADVD PKKVDQESDP


        251                                                       300
prta6   DVFSTYPMLR LHPWHRQRFY HKFQHLYAPF IFGFMTINKV ISQDVGVVLR
prta8   DVFSTYPMLR LHPWHRQRFY HKFQHLYAPF IFGSMTINKV ISQDVGVVLR
prta7   DVFSTYPMLR LHPWHRQRFY HKFQHLYAPL IFGFMTINKV ISQDVGVVLR
prta5   DVFSTYPMLR LHPWHRQRFY HKFQHLYAPF IFGFMTINKV ISQDVGVVLR
```

FIG.2A

Amino Acid Comparison of Delta-4 Desaturases

```
       301                                                    350
prta6  KRLFQIDANC RYGSPWYVAR FWIMKLLTTL YMVALPMYMQ GPAQGLKLFF
prta8  KRLFQIDANC RYGSPWYVAR FWIMKLLTTL YMVALPMYMQ GPAQGLKLFF
prta7  KRLFQIDANC RYGSPWNVAR FWIMKLLTTL YMVALPMYMQ GPAQGLKLFF
prta5  KRLFQIDANC RYGSPWYVAR FWIMKLLTTL YTVALPMYMQ GPAQGLKLFF


       351                                                    400
prta6. MAHFTCGEVL ATMFIVNHII EGVSYASKDA VKGVMAPPRT VHGVTPMQVT
prta8  MAHFTCGEVL ATMFIVNHII EGVSYASKDA VKGVMAPPRT VHGVTPMQVT
prta7  MAHFTCGEVL ATMFIVNHII EGVSYASKDA VKGVMAPPRT VHGVTPMQVT
prta5  MAHFTCGEVL ATMFIVNHII EGVSYASKDA VKGVMAPPRT VHGVTPMQVT


       401                                                    450
prta6  QKALSAAEST KSDADKTTMI PLNDWAAVQC QTSVNWAVGS WFWNHFSGGL
prta8  QKALSAAESA KSDADKTTMI PLNDWAAVQC.QTSVNWAVGS WFWNHFSGGL
prta7  QKALSAAEST KSDADKTTMI PLNDWAAVQC QTSVNWAVGS WFWNHFSGGL
prta5  QKALSAAEST KSDADKTTMI PLNDWAAVQC QTSVNWAVGS WFWNHFSGGL


       451                                                    500
prta6  NHQIEHHCFP QNPHTVNVYI SGIVKETCEE YGVPYQAEIS LFSAYFKMLS
prta8  NHQIEHHCFP QNPHTVNVYI SGIVKETCEE YGVPYQAEIS LFSAYFKMLS
prta7  NHQIEHHCFP QNPHTVNVYI SGIVKETCEE YGVPYQAEIS LFSAYFKMLS
prta5  NHQIEHHCFP QNPHTVNVYI SGIVKETCEE YGVPYQAEIS LFSAYFKMLS


       500
prta6  HLRTLGNEDL TAWST*
prta8  HLRTLGNEDL TAWST*
prta7  HLRTLGNEDL TAWST*
prta5  HLRTLGNEDL TARST*
```

FIG.2B

Gene Sequence of Delta 4-Desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA5

```
atgacggtcg ggtttgacga aacggtgact atggacacgg tccgcaacca caacatgccg   60
gacgacgcct ggtgcgcgat ccacggcacc gtgtacgaca tcaccaagtt cagcaaggtg  120
cacccgcccg gggacatcat catgctggcc gctggcaagg aggccaccat cctgttcgag  180
acgtaccaca tcaagggcgt cccggacgcg gtgctgcgca agtacaaggt cggcaagctc  240
cccccaggca agaagggcga aacgagccac atgcccaccg ggctcgactc ggcctcctac  300
tactcgtggg acagcgagtt ttacagggtg ctccgcgagc ggtcgcccaa gaagctggcc  360
gagcccgccc tcatgcagcg cgcgcgcatg gagctctggg ccaaggcgat cttcctcctg  420
gcaggtttct gggctcccct ttacgctcgg tgcgtgctag accgcacggg cggtgccatg  480
gtagccgccg ttacgtccgg cgtgttcgct gcctttgtcg gaacttgcat ccagcacgac  540
ggcagccacg gcgccttctc caagtcgcga ttcatgaaca aggcggcggg ctggaccctc  600
gacatgatcg gcgcgagcgc gatgacctgg gagatgcagc acgttcttgg ccaccacccg  660
tacaccaacc tcatcgagat ggagaacggt ttggccaagg tcaagggcga cgacgtcgac  720
ccgaagaagg tcgaccagga gagcgacccg gacgtcttca gtacgtaccc gatgcttcgc  780
ctgcacccgt ggcaccgcca gcggttttac cacaagttcc agcacctgta cgccccgttt  840
atctttgggt ttatgacgat taacaaggtg atttcccagg atgtcggggt tgtgctgcgc  900
aagcgcctgt tccagatcga cgccaactgc cggtatggca gcccctggta cgtggcccgc  960
ttctgatcca tgaagctcct caccacgctc tacacggtgg cgcttcccat gtacatgcag 1020
gggctgctcc agggcttgaa gcttttcttc atggcccact tcacctgcgg agaggtcctc 1080
gccaccatgt ttattgtcaa ccacatcatc gagggcgtca gctacgcttc caaggacgcg 1140
gtcaagggcg tcatggctcc gccgcgcact gtgcacggtg tcaccccgat gcaggtgacg 1200
caaaaggcgc tcagtggcgc cgagtcgacc aagtcggacg ccgacaagac gaccatgatc 1260
ccctcaaccg actgggccgc tgtcagtggc cagacctctg tgaactgggc tgtcgggtcg 1320
tggttttgga accactttc gggcggcctc aaccaccaga ttgagcacca ctgcttcccc 1380
caaaacccc acacggtcaa cgtctacatc tcgggcatcg tcaaggagac ctgcgaagaa 1440
tacggcgtgc cgtaccaggc tgagatcagc cgaggacctc cctatttctg gatgctgtcg 1500
cacctccgca cgctcggcaa acggccaggt cgaggacctc ccacgtga            1548
```

FIG.3

Gene Sequence of Delta 4-Desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA6

```
atgacggtcg ggtttgacga aacggtgact atggacacgg tccgcaacca caacatgccg   60
gacgacgcct ggtgcgcgat ccacggcacc gtgtacgaca tcaccaagtt cagcaaggtg  120
cacccggcg gggacatcat catgctggcc gctggcaagg aggccaccat cctgttcgag  180
agtaccaca tcaagggcgt tccggacgcg gtgctgcgca agtacaaggt cggcaagctc  240
cccagggca agaagggcga aacgagccac atgcccaccg ggctcgactc ggccttctac  300
tactcgtggg acagcgagtt ttacagggtg ctccgcgagc ggtcgccaa gaagctggcc  360
gagcccggcc tcatgcagcg cgcgcgcatg gagctctggg ccaaggcgat cttcctcctg  420
gcaggtttct ggggctccct ttacgccatg tgcgtgctag accgcacgg cggtgccatg  480
gtagccgccg ttacgctcgg cgtgttcgct gcctttgtcg gaacttgcat ccagcacgac  540
ggcagccacg gcgccttctc caagtcgcga ttcatgaaca agcggcggg ctggaccctc  600
gacatgatcg gcgcgagcgc gatgacctgg gagatgcagc acgttcttgg ccaccacccg  660
tacaccaacc tcatcgagat ggagaacggt ttggccaagg tcaagggcgc cgacgtcgac  720
ccgaagaagg tcgaccagga gagcgacccg gacgtcttca gtacgtaccc gatgcttcgc  780
ctgcacccgt ggcaccgcca gcggttttac cacaagttcc agcacctgta cgccccgttt  840
atctttgggt ttatgacgat taacaaggtg atttcccagg atgtcggggt tgtgctgcgc  900
aagcgcctgt tccagatcga cgccaactgc cggtatggca gcccctggta cgtggcccgc  960
ttctggatca tgaagctcct caccacgctc tacatggtgg cgcttcccat gtacatgcag 1020
gggctgctc aggcttgaa gctttttctc atggccccact tcacctgcgg agaggtcctc 1080
gccaccatgt ttattgtcaa ccacatcatc gagggggtca gctacgcttc caaggacgcg 1140
gtcaagggcg tcatggctcc gccgcgcact gtgcacggtg tcaccccgat gcaggtgacg 1200
caaaaggcgc tcagtgcggc cgagtcgacc aagtcggacg ccgacaagac gaccatgatc 1260
cccctcaacg actgggccgc tgtgcagtgc cagacctctg tgaactgggc tgtcgggtcg 1320
tggttttgga accactttc gggcggcctc aaccaccaga ttgagcacca ctgcttcccc 1380
caaaacccc acacggtcaa cgtctacatc tcaggcatcg tcaaggagac ctgcgaagaa 1440
tacggctgc cgtaccaggc tgagatcagc ctcttctctg tcaaggagac gatgctgtcg 1500
cacctccgca cgctcggcaa cgaggacctc acggcctggt ccacgtga           1548
```

FIG.4

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Gene Sequence of Delta 4-Desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA7

```
atgacggtcg ggtttgacga aacggtgact atggacacgg tccgcaacca caacatgccg    60
gacgacgcct ggtgcgcgat ccacggcacc gtgtacgaca tcaccaagtt cagcaaggtg   120
cacccgcgcg gggacatcat catgctggcc gctggcaagg aggccaccat cctgttcgag   180
acctaccaca tcaagggcgt cccggacgcg gtgctgcgca agtacaaggt cggcaagctc   240
cccaggcga agaagggcga aacgagccac atgcccaccg ggctcgactc ggcctcctac   300
tactcgtggg acagcgagtt ttacaggtg ctccgcgagc ggtcgccaa gaagctggcc   360
gagcccggcc tcatgcagcg cgcgcgcatg gagctctggg ccaaggcgat cttcctcctg   420
gcaggtttct ggggctccct ttacgccatg tgcgtgctag acccgcacgg cggtgccatg   480
gtagccgccg ttacgctcgg cgtgttcgct gcctttgtcg gaacttgcat ccagcacgac   540
ggcagccacg gcgccttctc caagtcgcga ttcatgaaca agcggcggg ctggaccctc   600
gacatgatcg gcgcgagcgc gatgacctgg gagatgcagc acgttcttgg tcaccacccg   660
tacaccaacc tcatcgagat ggagaacggt ttggccaagg tcaagggcgc cgacgtcgac   720
ccgaagaagg tcgaccagga gagcgacccg gacgtcttca gtacgtaccc gatgcttcgc   780
ctgcacccgt ggcaccgcca gcggttttac cacaagttcc agcacctgta cgccccgctt   840
atctttgggt ttatgacgat taacaaggtg atttcccagg atgtcggggt tgtgctgcgc   900
aagcgcctgt tccagatcga cgccaactgc cggtatggca gccccctgaa cgtggcccgc   960
ttctggatca tgaagctcct caccacgctc tacatggtgg cgcttcccat gtacatgcag  1020
gggctgctc agggcttgaa gctttttttc ccacatcatc atggccccact tcacctgcgg agaggtcctc  1080
gccaccatgt ttattgtcaa ccacatcatc gagggcgtca gctacgcttc caaggacgcg  1140
gtcaagggcg tcatggctcc gccgcgcact gtgcacggtg tcaccccgat gcaggtgacg  1200
caaaaggcgc tcagtgcggc cgagtcgacc aagtcggacg ccgacaagac gaccatgatc  1260
cccctcaacg actgggccgc tgtgcagtgc cagacctctg tgaactgggc tgtcgggtcg  1320
tggttttgga accactttc gggcggccta aaccaccaga ttgagcacca ctgcttcccc  1380
caaaaccccc acacggtcaa cgtctacatc tcgggcatcg tcaaggagac ctgcgaagaa  1440
tacggcgtgc cgtaccaggc tgagatcagc ctcttctctg cctatttcaa gatgctgtcg  1500
cacctccgca cgctcggcaa cgaggacctc acggcctggt ccacgtga             1548
```

FIG.5

Gene Sequence of Delta 4-Desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA8

```
atgacggtcg  ggtttgacga  aacggtgact  atggacacgg  tccgcaacca  caacatgccg     60
gacgacgcct  ggtgcgcgat  ccacggcacc  gtgtacgaca  tcaccaagtt  cagcaaggtg    120
cacccggcg   gggacatcat  catgctggcc  gctggcaagg  aggccaccat  cctgttcgag    180
acctaccaca  tcaagggcgt  cccgacgcg   gtgctgcgca  agtacaaggt  cggcaagctc    240
cccaggca    agaagggcga  aacgagccac  atgcccaccg  ggctcgactc  ggcctcctac    300
tactcgtggg  acagcgagtt  ttacagggtg  ctccgcgagc  gcgtcgccaa  gaagctggcc    360
gagcccggcc  tcatgcagcg  cgcgcgcatg  gagctctggg  ccaaggcgat  cttcctcctg    420
gcaggtttct  gggctccct   ttacgccatg  tgcgtgctag  accgcacgg   cggtgccatg    480
gtagccgccg  ttacgctcgg  cgtgttcgct  gcctttgtcg  gaacttgcat  ccagcacgac    540
ggcagccacg  gcgccttctc  caagtcgcga  ttcatgaaca  aggcgggcgg  ctggaccctc    600
gacatgatcg  gcgcgagtgc  gatgacctgg  gagatgcagc  acgttcttgg  ccaccaccg    660
tacaccaacc  tcatcgagat  ggagaacggt  ttggccaagg  tcaagggcgc  cgacgtcgac    720
ccgaagaagg  tcgaccagga  gagcgacccg  gacgtcttca  gtacgtaccc  gatgcttcgc    780
ctgcacccgt  ggcaccgcca  gcggtttac   cacaagttcc  agcacctgta  cgccccgttt    840
atctttgggt  ctatgacgat  taacaaggtg  atttcccagg  atgtcggggt  tgtgctgcgc    900
aagcgcctgt  tccagatcga  cgccaactgc  cggtatgca   gccccctggta  cgtggcccgc    960
ttcttgatca  tgaagctcct  caccacgctc  tacatggtgg  cgcttccat   gtacatgcag   1020
gggcctgctc  aggcttgaa   gctttcttc   ccacatcatc  atggcccact  tcacctgcgg  agaggtcctc  1080
gccaccatgt  ttattgtcaa  ccacatcatc  gagggcgtca  gctacgcttc  caaggacgcg   1140
gtcaaggcg   tcatggctcc  gccgcgcact  gtgcacggtg  tcaccccgat  gcaggtgacg   1200
caaaggcgc   tcagtcggc   cgagtcggcc  aagtcggacg  ccgacaagac  gaccatgatc   1260
cccctcaacg  actgggccgc  tgtgcagtgc  cagacctctg  tgaactgggc  tgtcgggtcg   1320
tggttttgga  accacttttc  gggcgcctc   aaccaccaga  ttgagcacca  ctgcttcccc   1380
caaaacccc   acacggtcaa  cgtctacatc  tcgggcatcg  tcaaggagac  ctgcgaagaa   1440
tacggcgtgc  cgtaccagc   tgagatcagc  cgaggacctc  cctatttcaa  gatgctgtcg   1500
caccttccgca  cgctcggcaa  cgaggacctc  acggcctggt  ccacgtga                 1548
```

FIG.6

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Gene Sequence of Delta 4-Desaturase of *Thraustochytrium aureum*
(ATCC 34304) from plasmid pRTA5

```
Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn
 1           5               10              15
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
        20              25                      30
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
        35              40                      45
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
    50              55                  60
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu
65              70                  75                      80
Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp
            85                  90                      95
Ser Ala Ser Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg
            100             105                 110
Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala
        115             120                 125
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
    130             135                 140
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met
145             150                 155                     160
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys
                165                 170                 175
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
            180                 185                 190
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
        195                 200                 205
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu
    210                 215                 220
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
225                 230                 235                     240
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
                245                 250                 255
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
            260                 265                 270
Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Phe Met Thr Ile Asn
        275                 280                 285
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
    290                 295                 300
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg
305                 310                 315                     320
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Thr Val Ala Leu Pro
                325                 330                 335
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
            340                 345                 350
```

FIG.7A

```
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
        355                 360                 365
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
    370                 375                 380
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385                 390                 395                     400
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys
            405                 410                     415
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
            420                 425                 430
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
        435                 440                 445
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
    450                 455                 460
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
465                 470                 475                     480
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
            485                 490                     495
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
            500                 505                 510
Arg Ser Thr
            515
```

FIG.7B

Gene Sequence of Delta 4-Desaturase of *Thraustochytrium aureum* (ATCC 34304) from plasmid pRTA6

```
Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn
 1           5                   10                  15
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
            20                  25                  30
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
        35                  40                  45
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
    50                  55                  60
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu
65                  70                  75                  80
Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp
            85                  90                  95
Ser Ala Phe Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg
            100                 105                 110
Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala
        115                 120                 125
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
    130                 135                 140
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met
145                 150                 155                 160
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys
            165                 170                 175
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
            180                 185                 190
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
        195                 200                 205
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu
    210                 215                 220
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
225                 230                 235                 240
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
            245                 250                 255
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
            260                 265                 270
Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Phe Met Thr Ile Asn
        275                 280                 285
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
    290                 295                 300
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg
305                 310                 315                 320
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro
            325                 330                 335
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
            340                 345                 350
```

FIG.8A

```
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
        355             360             365
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
    370             375             380
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385             390             395             400
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys
            405             410             415
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
            420             425             430
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
        435             440         .       445
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
    450             455             460
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
465             470             475             480
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
            485             490             495
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
            500             505             510
Trp Ser Thr
        515
```

FIG.8B

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Gene Sequence of Delta 4-Desaturase of *Thraustochytrium aureum*
(ATCC 34304) from plasmid pRTA7

```
Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn
 1           5                   10                  15
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
            20                  25                  30
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
        35                  40                  45
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
    50                  55                  60
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu
65                  70                  75                  80
Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp
            85                  90                  95
Ser Ala Ser Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg
            100                 105                 110
Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala
            115                 120                 125
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
    130                 135                 140
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met
145                 150                 155                 160
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys
            165                 170                 175
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
            180                 185                 190
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
            195                 200                 205
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu
    210                 215                 220
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
225                 230                 235                 240
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
            245                 250                 255
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
            260                 265                 270
Phe Gln His Leu Tyr Ala Pro Leu Ile Phe Gly Phe Met Thr Ile Asn
    275                 280                 285
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
    290                 295                 300
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Asn Val Ala Arg
305                 310                 315                 320
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro
            325                 330                 335
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
            340                 345                 350
```

FIG.9A

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```
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
        355             360             365
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
    370             375             380
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385             390             395             400
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys
            405             410             415
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
        420             425             430
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
        435             440             445
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
    450             455             460
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
465             470             475             480
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
            485             490             495
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
        500             505             510
Trp Ser Thr
        515
```

FIG.9B

Gene Sequence of Delta 4-Desaturase of *Thraustochytrium aureum*
(ATCC 34304) from plasmid pRTA8

```
Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn
 1           5               10              15
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
        20              25                      30
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
        35              40                      45
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
    50              55                  60
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu
65              70                  75                      80
Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp
            85              90                      95
Ser Ala Ser Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg
            100             105             110
Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala
        115             120                 125
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
    130             135             140
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met
145             150             155             160
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys
            165             170             175
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
        180             185             190
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
        195             200             205
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu
    210             215             220
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
225             230             235             240
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
            245             250             255
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
            260             265             270
Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Ser Met Thr Ile Asn
        275             280             285
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
    290             295             300
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg
305             310             315             320
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro
            325             330             335
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
            340             345             350
```

FIG.10A

```
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
        355             360             365
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
    370             375             380
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385             390             395             400
Gln Lys Ala Leu Ser Ala Ala Glu Ser Ala Lys Ser Asp Ala Asp Lys
            405             410             415
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
            420             425             430
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
        435             440             445
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
    450             455             460
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
465             470             475             480
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
            485             490             495
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
            500             505             510
Trp Ser Thr
        515
```

FIG.10B

SEQUENCE ID NO:1
5'-GTBTAYGAYGTBACCGARTGGGTBAAGCGYCAYCCBGGHGGH-3'

SEQUENCE ID NO:2
5'-GGHGCYTCCGCYAACTGGTGGAAGCAYCAGCAYAACGTBCAYCAY-3'

SEQUENCE ID NO:3
5'-RTGRTGVACGTTRTGCTGRTGCTTCCACCAGTTRGCGGARGCDCC-3'

SEQUENCE ID NO:4
5'-TTGATRGTCTARCTYGTRGTRGASAARGGVTGGTAC-3'

SEQUENCE ID NO:5
5'-CATCATCATXGGRAAXARRTGRTG-3'

SEQUENCE ID NO:6
5'-CTACTACTACTACAYCAYACXTAYACXAAY-3'

SEQUENCE ID NO:7
5'-CCCAGTCACGACGTTGTAAAACGACGGCCAG-3'

SEQUENCE ID NO:8
5'-GACGATTAACAAGGTGATTTCCCAGGATGTC

SEQUENCE ID NO:9
5'-GACTAACTCGAGTCACGTGGACCAGGCCGTGAGGTCCT

FIG.11A

SEQUENCE ID NO:10
GACTAACTCGAGTTGACGAGGTTTGTATGTTCGGCGGTTTGCTTG-3'

SEQUENCE ID NO:11
5'-AGCGGATAACAATTTCACACAGGAAACAGC-3'

SEQUENCE ID NO:12
TGGCTACCGTCGTGCTGGATGCAAGTTCCG-3'

SEQUENCE ID NO:13
5'-CGCATGGAATTCATGACGGTCGGGTTTGACGAAACGGTG-3'

SEQUENCE ID NO:14

```
   1 ATGACGGTCG GGTTTGACGA AACGGTGACT ATGGACACGG TCCGCAACCA
  51 CAACATGCCG GACGACGCCT GGTGCGCGAT CCACGGCACC GTGTACGACA
 101 TCACCAAGTT CAGCAAGGTG CACCCCGGCG GGGACATCAT CATGCTGGCC
 151 GCTGGCAAGG AGGCCACCAT CCTGTTCGAG ACGTACCACA TCAAGGGCGT
 201 CCCGGACGCG GTGCTGCGCA AGTACAAGGT CGGCAAGCTC CCCCAGGGCA
 251 AGAAGGGCGA AACGAGCCAC ATGCCCACCG GGCTCGACTC GGCCTCCTAC
 301 TACTCGTGGG ACAGCGAGTT TTACAGGGTG CTCCGCGAGC GCGTCGCCAA
 351 GAAGCTGGCC GAGCCCGGCC TCATGCAGCG CGCGCGCATG GAGCTCTGGG
 401 CCAAGGCGAT CTTCCTCCTG GCAGGTTTCT GGGGCTCCCT TTACGCCATG
 451 TGCGTGCTAG ACCCGCACGG CGGTGCCATG GTAGCCGCCG TTACGCTCGG
 501 CGTGTTCGCT GCCTTTGTCG GAACTTGCAT CCAGCACGAC GGCAGCCACG
 551 GCGCCTTCTC CAAGTCGCGA TTCATGAACA AGGCGGCGGG CTGGACCCTC
 601 GACATGATCG GCGCGAGCGC GATGACCTGG GAGATGCAGC ACGTTCTTGG
 651 CCACCACCCG TACACCAACC TCATCGAGAT GGAGAACGGT TTGGCCAAGG
 701 TCAAGGGCGC CGACGTCGAC CCGAAGAAGG TCGACCAGGA GAGCGACCCG
 751 GACGTCTTCA GTACGTACCC GATGCTTCGC CTGCACCCGT GGCACCGCCA
 801 GCGGTTTTAC CACAAGTTCC AGCACCTGTA CGCCCCGTTT ATCTTTGGGT
 851 TTATGACGAT TAACAAGGTG ATTTCCCAGG ATGTCGGGGT TGTGCTGCGC
 901 AAGCGCCTGT TCCAGATCGA CGCCAACTGC CGGTATGGCA GCCCCTGGTA
 951 CGTGGCCCGC TTCTGGATCA TGAAGCTCCT CACCCACGCTC TACACGGTGG
1001 CGCTTCCCAT GTACATGCAG GGGCCTGCTC AGGGCTTGAA GCTTTTCTTC
1051 ATGGCCCACT TCACCTGCGG AGAGGTCCTC GCCACCATGT TTATTGTCAA
```

FIG.11B

```
1101 CCACATCATC GAGGGCGTCA GCTACGCTTC CAAGGACGCG GTCAAGGGCG
1151 TCATGGCTCC GCCGCGCACT GTGCACGGTG TCACCCCGAT GCAGGTGACG
1201 CAAAAGGCGC TCAGTGCGGC CGAGTCGACC AAGTCGGACG CCGACAAGAC
1251 GACCATGATC CCCCTCAACG ACTGGGCCGC TGTGCAGTGC CAGACCTCTG
1301 TGAACTGGGC TGTCGGGTCG TGGTTTTGGA ACCACTTTTC GGGCGGCCTC
1351 AACCACCAGA TTGAGCACCA CTGCTTCCCC CAAAACCCCC ACACGGTCAA
1401 CGTCTACATC TCGGGCATCG TCAAGGAGAC CTGCGAAGAA TACGGCGTGC
1451 CGTACCAGGC TGAGATCAGC CTCTTCTCTG CCTATTTCAA GATGCTGTCG
1501 CACCTCCGCA CGCTCGGCAA CGAGGACCTC ACGGCCAGGT CCACGTGA
```


SEQUENCE ID NO:15
```
   1 ATGACGGTCG GGTTTGACGA AACGGTGACT ATGGACACGG TCCGCAACCA
  51 TAACATGCCG GACGACGCCT GGTGCGCGAT CCACGGCACC GTGTACGACA
 101 TCACCAAGTT CAGCAAGGTG CACCCCGGCG GGGACATCAT CATGCTGGCC
 151 GCTGGCAAGG AGGCCACCAT CCTGTTCGAG ACGTACCACA TCAAGGGCGT
 201 CCCGGACGCG GTGCTGCGCA AGTACAAGGT CGGCAAGCTC CCCCAGGGCA
 251 AGAAGGGCGA AACGAGCCAC ATGCCCACCG GGCTCGACTC GGCCTTCTAC
 301 TACTCGTGGG ACAGCGAGTT TTACAGGGTG CTCCGCGAGC GCGTCGCCAA
 351 GAAGCTGGCC GAGCCCGGCC TCATGCAGCG CGCGCGCATG GAGCTCTGGG
 401 CCAAGGCGAT CTTCCTCCTG GCAGGTTTCT GGGGCTCCCT TTACGCCATG
 451 TGCGTGCTAG ACCCGCACGG CGGTGCCATG GTAGCCGCCG TTACGCTCGG
 501 CGTGTTCGCT GCCTTTGTCG GAACTTGCAT CCAGCACGAC GGCAGCCACG
 551 GCGCCTTCTC CAAGTCGCGA TTCATGAACA AGGCGGCGGG CTGGACCCTC
 601 GACATGATCG GCGCGAGCGC GATGACCTGG GAGATGCAGC ACGTTCTTGG
 651 CCACCACCCG TACACCAACC TCATCGAGAT GGAGAACGGT TTGGCCAAGG
 701 TCAAGGGCGC CGACGTCGAC CCGAAGAAGG TCGACCAGGA GAGCGACCCG
 751 GACGTCTTCA GTACGTACCC GATGCTTCGC CTGCACCCGT GGCACCGCCA
 801 GCGGTTTTAC CACAAGTTCC AGCACCTGTA CGCCCCGTTT ATCTTTGGGT
 851 TTATGACGAT TAACAAGGTG ATTTCCCAGG ATGTCGGGGT TGTGCTGCGC
 901 AAGCGCCTGT TCCAGATCGA CGCCAACTGC CGGTATGGCA GCCCCTGGTA
 951 CGTGGCCCGC TTCTGGATCA TGAAGCTCCT CACCCACGCTC TACATGGTGG
1001 CGCTTCCCAT GTACATGCAG GGGCCTGCTC AGGGCTTGAA GCTTTTCTTC
1051 ATGGCCCACT TCACCTGCGG AGAGGTCCTC GCCACCATGT TTATTGTCAA
1101 CCACATCATC GAGGGCGTCA GCTACGCTTC CAAGGACGCG GTCAAGGGCG
1151 TCATGGCTCC GCCGCGCACT GTGCACGGTG TCACCCCGAT GCAGGTGACG
1201 CAAAAGGCGC TCAGTGCGGC CGAGTCGACC AAGTCGGACG CCGACAAGAC
1251 GACCATGATC CCCCTCAACG ACTGGGCCGC TGTGCAGTGC CAGACCTCTG
1301 TGAACTGGGC TGTCGGGTCG TGGTTTTGGA ACCACTTTTC GGGCGGCCTC
1351 AACCACCAGA TTGAGCACCA CTGCTTCCCC CAAAACCCCC ACACGGTCAA
1401 CGTCTACATC TCGGGCATCG TCAAGGAGAC CTGCGAAGAA TACGGCGTGC
1451 CGTACCAGGC TGAGATCAGC CTCTTCTCTG CCTATTTCAA GATGCTGTCG
1501 CACCTCCGCA CGCTCGGCAA CGAGGACCTC ACGGCCTGGT CCACGTGA
```

FIG.11C

SEQUENCE ID NO:16

```
   1 ATGACGGTCG GGTTTGACGA AACGGTGACT ATGGACACGG TCCGCAACCA
  51 CAACATGCCG GACGACGCCT GGTGCGCGAT CCACGGCACC GTGTACGACA
 101 TCACCAAGTT CAGCAAGGTG CACCCCGGCG GGGACATCAT CATGCTGGCC
 151 GCTGGCAAGG AGGCCACCAT CCTGTTCGAG ACCTACCACA TCAAGGGCGT
 201 CCCGGACGCG GTGCTGCGCA AGTACAAGGT CGGCAAGCTC CCCCAGGGCA
 251 AGAAGGGCGA AACGAGCCAC ATGCCCACCG GGCTCGACTC GGCCTCCTAC
 301 TACTCGTGGG ACAGCGAGTT TTACAGGGTG CTCCGCGAGC GCGTCGCCAA
 351 GAAGCTGGCC GAGCCCGGCC TCATGCAGCG CGCGCGCATG GAGCTCTGGG
 401 CCAAGGCGAT CTTCCTCCTG GCAGGTTTCT GGGGCTCCCT TTACGCCATG
 451 TGCGTGCTAG ACCCGCACGG CGGTGCCATG GTAGCCGCCG TTACGCTCGG
 501 CGTGTTCGCT GCCTTTGTCG GAACTTGCAT CCAGCACGAC GGCAGCCACG
 551 GCGCCTTCTC CAAGTCGCGA TTCATGAACA AGGCGGCGGG CTGGACCCTC
 601 GACATGATCG GCGCGAGCGC GATGACCTGG GAGATGCAGC ACGTTCTTGG
 651 TCACCACCCG TACACCAACC TCATCGAGAT GGAGAACGGT TTGGCCAAGG
 701 TCAAGGGCGC CGACGTCGAC CCGAAGAAGG TCGACCAGGA GAGCGACCCG
 751 GACGTCTTCA GTACGTACCC GATGCTTCGC CTGCACCCGT GGCACCGCCA
 801 GCGGTTTTAC CACAAGTTCC AGCACCTGTA CGCCCCGCTT ATCTTTGGGT
 851 TTATGACGAT TAACAAGGTG ATTTCCCAGG ATGTCGGGGT TGTGCTGCGC
 901 AAGCGCCTGT TCCAGATCGA CGCCAACTGC CGGTATGGCA GCCCCTGGAA
 951 CGTGGCCCGC TTCTGGATCA TGAAGCTCCT CACCACGCTC TACATGGTGG
1001 CGCTTCCCAT GTACATGCAG GGGCCTGCTC AGGGCTTGAA GCTTTTCTTC
1051 ATGGCCCACT TCACCTGCGG AGAGGTCCTC GCCACCATGT TTATTGTCAA
1101 CCACATCATC GAGGGCGTCA GCTACGCTTC CAAGGACGCG GTCAAGGGCG
1151 TCATGGCTCC GCCGCGCACT GTGCACGGTG TCACCCCGAT GCAGGTGACG
1201 CAAAAGGCGC TCAGTGCGGC CGAGTCGACC AAGTCGGACG CCGACAAGAC
1251 GACCATGATC CCCCTCAACG ACTGGGCCGC TGTGCAGTGC CAGACCTCTG
1301 TGAACTGGGC TGTCGGGTCG TGGTTTTGGA ACCACTTTTC GGGCGGCCTA
1351 AACCACCAGA TTGAGCACCA CTGCTTCCCC CAAAACCCCC ACACGGTCAA
1401 CGTCTACATC TCGGGCATCG TCAAGGAGAC CTGCGAAGAA TACGGCGTGC
1451 CGTACCAGGC TGAGATCAGC CTCTTCTCTG CCTATTTCAA GATGCTGTCG
1501 CACCTCCGCA CGCTCGGCAA CGAGGACCTC ACGGCCTGGT CCACGTGA
```

SEQUENCE ID NO:17

```
   1 ATGACGGTCG GGTTTGACGA AACGGTGACT ATGGACACGG TCCGCAACCA
  51 CAACATGCCG GACGACGCCT GGTGCGCGAT CCACGGCACC GTGTACGACA
 101 TCACCAAGTT CAGCAAGGTG CACCCCGGCG GGGACATCAT CATGCTGGCC
 151 GCTGGCAAGG AGGCCACCAT CCTGTTCGAG ACCTACCACA TCAAGGGCGT
 201 CCCGGACGCG GTGCTGCGCA AGTACAAGGT CGGCAAGCTC CCCCAGGGCA
 251 AGAAGGGCGA AACGAGCCAC ATGCCCACCG GGCTCGACTC GGCCTCCTAC
 301 TACTCGTGGG ACAGCGAGTT TTACAGGGTG CTCCGCGAGC GCGTCGCCAA
 351 GAAGCTGGCC GAGCCCGGCC TCATGCAGCG CGCGCGCATG GAGCTCTGGG
```

FIG.11D

```
 401 CCAAGGCGAT CTTCCTCCTG GCAGGTTTCT GGGGCTCCCT TTACGCCATG
 451 TGCGTGCTAG ACCCGCACGG CGGTGCCATG GTAGCCGCCG TTACGCTCGG
 501 CGTGTTCGCT GCCTTTGTCG GAACTTGCAT CCAGCACGAC GGCAGCCACG
 551 GCGCCTTCTC CAAGTCGCGA TTCATGAACA AGGCGGCGGG CTGGACCCTC
 601 GACATGATCG GCGCGAGTGC GATGACCTGG GAGATGCAGC ACGTTCTTGG
 651 CCACCACCCG TACACCAACC TCATCGAGAT GGAGAACGGT TTGGCCAAGG
 701 TCAAGGGCGC CGACGTCGAC CCGAAGAAGG TCGACCAGGA GAGCGACCCG
 751 GACGTCTTCA GTACGTACCC GATGCTTCGC CTGCACCCGT GGCACCGCCA
 801 GCGGTTTTAC CACAAGTTCC AGCACCTGTA CGCCCCGTTT ATCTTTGGGT
 851 CTATGACGAT TAACAAGGTG ATTTCCCAGG ATGTCGGGGT TGTGCTGCGC
 901 AAGCGCCTGT TCCAGATCGA CGCCAACTGC CGGTATGGCA GCCCCTGGTA
 951 CGTGGCCCGC TTCTGGATCA TGAAGCTCCT CACCACGCTC TACATGGTGG
1001 CGCTTCCCAT GTACATGCAG GGGCCTGCTC AGGGCTTGAA GCTTTTCTTC
1051 ATGGCCCACT TCACCTGCGG AGAGGTCCTC GCCACCATGT TTATTGTCAA
1101 CCACATCATC GAGGGCGTCA GCTACGCTTC CAAGGACGCG GTCAAGGGCG
1151 TCATGGCTCC GCCGCGCACT GTGCACGGTG TCACCCCGAT GCAGGTGACG
1201 CAAAAGGCGC TCAGTGCGGC CGAGTCGGCC AAGTCGGACG CCGACAAGAC
1251 GACCATGATC CCCCTCAACG ACTGGGCCGC TGTGCAGTGC CAGACCTCTG
1301 TGAACTGGGC TGTCGGGTCG TGGTTTTGGA ACCACTTTTC GGGCGGCCTC
1351 AACCACCAGA TTGAGCACCA CTGCTTCCCC CAAAACCCCC ACACGGTCAA
1401 CGTCTACATC TCGGGCATCG TCAAGGAGAC CTGCGAAGAA TACGGCGTGC
1451 CGTACCAGGC TGAGATCAGC CTCTTCTCTG CCTATTTCAA GATGCTGTCG
1501 CACCTCCGCA CGCTCGGCAA CGAGGACCTC ACGGCCTGGT CCACGTGA
```

SEQUENCE ID NO:18

```
   1 MTVGFDETVT MDTVRNHNMP DDAWCAIHGT VYDITKFSKV HPGGDIIMLA
  51 AGKEATILFE TYHIKGVPDA VLRKYKVGKL PQGKKGETSH MPTGLDSASY
 101 YSWDSEFYRV LRERVAKKLA EPGLMQRARM ELWAKAIFLL AGFWGSLYAM
 151 CVLDPHGGAM VAAVTLGVFA AFVGTCIQHD GSHGAFSKSR FMNKAAGWTL
 201 DMIGASAMTW EMQHVLGHHP YTNLIEMENG LAKVKGADVD PKKVDQESDP
 251 DVFSTYPMLR LHPWHRQRFY HKFQHLYAPF IFGFMTINKV ISQDVGVVLR
 301 KRLFQIDANC RYGSPWYVAR FWIMKLLTTL YTVALPMYMQ GPAQGLKLFF
 351 MAHFTCGEVL ATMFIVNHII EGVSYASKDA VKGVMAPPRT VHGVTPMQVT
 401 QKALSAAEST KSDADKTTMI PLNDWAAVQC QTSVNWAVGS WFWNHFSGGL
 451 NHQIEHHCFP QNPHTVNVYI SGIVKETCEE YGVPYQAEIS LFSAYFKMLS
 501 HLRTLGNEDL TARST*
```

SEQUENCE ID NO:19

```
   1 MTVGFDETVT MDTVRNHNMP DDAWCAIHGT VYDITKFSKV HPGGDIIMLA
  51 AGKEATILFE TYHIKGVPDA VLRKYKVGKL PQGKKGETSH MPTGLDSAFY
```

FIG.11E

```
101 YSWDSEFYRV LRERVAKKLA EPGLMQRARM ELWAKAIFLL AGFWGSLYAM
151 CVLDPHGGAM VAAVTLGVFA AFVGTCIQHD GSHGAFSKSR FMNKAAGWTL
201 DMIGASAMTW EMQHVLGHHP YTNLIEMENG LAKVKGADVD PKKVDQESDP
251 DVFSTYPMLR LHPWHRQRFY HKFQHLYAPF IFGFMTINKV ISQDVGVVLR
301 KRLFQIDANC RYGSPWYVAR FWIMKLLTTL YMVALPMYMQ GPAQGLKLFF
351 MAHFTCGEVL ATMFIVNHII EGVSYASKDA VKGVMAPPRT VHGVTPMQVT
401 QKALSAAEST KSDADKTTMI PLNDWAAVQC QTSVNWAVGS WFWNHFSGGL
451 NHQIEHHCFP QNPHTVNVYI SGIVKETCEE YGVPYQAEIS LFSAYFKMLS
501 HLRTLGNEDL TAWST*
```

SEQUENCE ID NO:20
```
  1 MTVGFDETVT MDTVRNHNMP DDAWCAIHGT VYDITKFSKV HPGGDIIMLA
 51 AGKEATILFE TYHIKGVPDA VLRKYKVGKL PQGKKGETSH MPTGLDSASY
101 YSWDSEFYRV LRERVAKKLA EPGLMQRARM ELWAKAIFLL AGFWGSLYAM
151 CVLDPHGGAM VAAVTLGVFA AFVGTCIQHD GSHGAFSKSR FMNKAAGWTL
201 DMIGASAMTW EMQHVLGHHP YTNLIEMENG LAKVKGADVD PKKVDQESDP
251 DVFSTYPMLR LHPWHRQRFY HKFQHLYAPL IFGFMTINKV ISQDVGVVLR
301 KRLFQIDANC RYGSPWNVAR FWIMKLLTTL YMVALPMYMQ GPAQGLKLFF
351 MAHFTCGEVL ATMFIVNHII EGVSYASKDA VKGVMAPPRT VHGVTPMQVT
401 QKALSAAEST KSDADKTTMI PLNDWAAVQC QTSVNWAVGS WFWNHFSGGL
451 NHQIEHHCFP QNPHTVNVYI SGIVKETCEE YGVPYQAEIS LFSAYFKMLS
501 HLRTLGNEDL TAWST*
```

SEQUENCE ID NO:21
```
  1 MTVGFDETVT MDTVRNHNMP DDAWCAIHGT VYDITKFSKV HPGGDIIMLA
 51 AGKEATILFE TYHIKGVPDA VLRKYKVGKL PQGKKGETSH MPTGLDSASY
101 YSWDSEFYRV LRERVAKKLA EPGLMQRARM ELWAKAIFLL AGFWGSLYAM
151 CVLDPHGGAM VAAVTLGVFA AFVGTCIQHD GSHGAFSKSR FMNKAAGWTL
201 DMIGASAMTW EMQHVLGHHP YTNLIEMENG LAKVKGADVD PKKVDQESDP
251 DVFSTYPMLR LHPWHRQRFY HKFQHLYAPF IFGSMTINKV ISQDVGVVLR
301 KRLFQIDANC RYGSPWYVAR FWIMKLLTTL YMVALPMYMQ GPAQGLKLFF
351 MAHFTCGEVL ATMFIVNHII EGVSYASKDA VKGVMAPPRT VHGVTPMQVT
401 QKALSAAESA KSDADKTTMI PLNDWAAVQC QTSVNWAVGS WFWNHFSGGL
451 NHQIEHHCFP QNPHTVNVYI SGIVKETCEE YGVPYQAEIS LFSAYFKMLS
501 HLRTLGNEDL TAWST*
```

FIG.11F

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SEQUENCE ID NO:22
```
ATGGAGCAGC TGAAGGCCTT TGATAATGAA GTCAATGCTT TCTTGGACAA CATGTTTGGA    60
CCACGAGATT CTCGAGTTCG CGGGTGGTTC CTGCTGGACT CTTACCTTCC CACCTTCATC   120
CTCACCATCA CGTACCTGCT CTCGATATGG CTGGGTAACA AGTACATGAA GAACAGGCCT   180
GCTCTGTCTC TCAGGGGCAT CCTCACCTTG TATAACCTCG CAATCACACT TCTTTCTGCG   240
TATATGCTGG TGGAGCTCAT CCTCTCCAGC TGGGAAGGAG GTTACAACTT GCAGTGTCAG   300
AATCTCGACA GTGCAGGAGA AGGTGATGTC CGGGTAGCCA AGGTCTTGTG GTGGTACTAC   360
TTCTCCAAAC TAGTGGAGTT CCTGGACACG ATTTTCTTTG TTCTACGAAA AAAGACCAAT   420
CAGATCACCT TCCTTCATGT CTATCACCAC GCGTCCATGT TCAACATCTG GTGGTGTGTT   480
TTGAACTGGA TACCTTGTGG TCAAAGCTTC TTTGGACCCA CCCTGAACAG CTTTATCCAC   540
ATTCTCATGT ACTCCTACTA CGGCCTGTCT GTGTTCCCGT CCATGCACAA GTACCTTTGG   600
TGGAAGAAGT ACCTCACACA GGCTCAGCTG GTGCAGTTCG TACTCACCAT CACGCACACG   660
CTGAGTGCCG TGGTGAAGCC CTGTGGCTTC CCCTTTGGCT GTCTCATCTT CCAGTCTTCC   720
TATATGATGA CGCTGGTCAT CCTGTTCTTA AACTTCTATA TTCAGACATA CCGGAAAAAG   780
CCAGTGAAGA AAGAGCTGCA AGAGAAAGAA GTGAAGAATG GTTTCCCCAA AGCCCACTTA   840
ATTGTGGCTA ATGGCATGAC GGACAAGAAG GCTCAATAA                          879
```

SEQUENCE ID NO:23
```
Met Glu Gln Leu Lys Ala Phe Asp Asn Glu Val Asn Ala Phe Leu Asp
1               5                   10                  15
Asn Met Phe Gly Pro Arg Asp Ser Arg Val Arg Gly Trp Phe Leu Leu
                20                  25                  30
Asp Ser Tyr Leu Pro Thr Phe Ile Leu Thr Ile Thr Tyr Leu Leu Ser
            35                  40                  45
Ile Trp Leu Gly Asn Lys Tyr Met Lys Asn Arg Pro Ala Leu Ser Leu
    50                  55                  60
Arg Gly Ile Leu Thr Leu Tyr Asn Leu Ala Ile Thr Leu Leu Ser Ala
65                  70                  75                  80
Tyr Met Leu Val Glu Leu Ile Leu Ser Ser Trp Glu Gly Gly Tyr Asn
                85                  90                  95
Leu Gln Cys Gln Asn Leu Asp Ser Ala Gly Glu Gly Asp Val Arg Val
            100                 105                 110
Ala Lys Val Leu Trp Trp Tyr Tyr Phe Ser Lys Leu Val Glu Phe Leu
    115                 120                 125
Asp Thr Ile Phe Phe Val Leu Arg Lys Lys Thr Asn Gln Ile Thr Phe
130                 135                 140
Leu His Val Tyr His His Ala Ser Met Phe Asn Ile Trp Trp Cys Val
145                 150                 155                 160
Leu Asn Trp Ile Pro Cys Gly Gln Ser Phe Phe Gly Pro Thr Leu Asn
                165                 170                 175
Ser Phe Ile His Ile Leu Met Tyr Ser Tyr Tyr Gly Leu Ser Val Phe
                180                 185                 190
```

FIG.11G

```
Pro Ser Met His Lys Tyr Leu Trp Trp Lys Lys Tyr Leu Thr Gln Ala
        195                 200             205
Gln Leu Val Gln Phe Val Leu Thr Ile Thr His Thr Leu Ser Ala Val
        210             215                 220
Val Lys Pro Cys Gly Phe Pro Phe Gly Cys Leu Ile Phe Gln Ser Ser
225             230                 235                 240
Tyr Met Met Thr Leu Val Ile Leu Phe Leu Asn Phe Tyr Ile Gln Thr
            245             250                 255
Tyr Arg Lys Lys Pro Val Lys Lys Glu Leu Gln Glu Lys Glu Val Lys
            260             265                 270
Asn Gly Phe Pro Lys Ala His Leu Ile Val Ala Asn Gly Met Thr Asp
            275             280                 285
Lys Lys Ala Gln
        290
```

SEQUENCE ID NO:24
```
ccagtgtgct ggaattcagg tactactact acaccatact tacacgaacc tgatcgagat    60
ggagaacggc acccaaaagg tcacccacgc cgacgtcgac cccaagaagg ccgaccagga   120
gagcgacccg gacgtcttca gcacctaccc catgctccgt ctgcacccgt ggcaccgcaa   180
gcgcttctac caccgcttcc agcacctgta cgcgccgctg ctcttcggtt tcatgaccat   240
caacaaggtg atcacccagg atgtgggagt tgtcctcagc aagcgtctgt ttcagatcga   300
tgccaactgc cgttacgcca gcaagtcgta cgttgcgcgc ttctggatca tgaagctgct   360
caccgtcctc tacatggtcg ccctccccgt gtacacccag ggccttgtcg acgggctcaa   420
gctcttcttc atcgcccact tttcgtgcgg cgagctgctg gccaccatgt tcatcgtcaa   480
ccacatcatc gagggcgtct cgtacgcctc caaggactct gtcaagggca ccatggcgcc   540
gccgcgcacg gtgcacggcg tgaccccgat gcatgacacc cgcgacgcgc tcggcaagga   600
gaaggcagcc accaagcacg tgccgctcaa cgactgggcc gcggtccagt gccagacctc   660
ggtcaactgg tcgatcggct cgtggttctg gaaccacttc tccggcgggc tcaaccacca   720
gatcgagcac cacctttttcc ccatgatgat gatg                               754
```

SEQUENCE ID NO:25
```
Gln Cys Ala Gly Ile Gln Val Leu Leu Leu His His Thr Tyr Thr Asn
1               5                 10                  15
Leu Ile Glu Met Glu Asn Gly Thr Gln Lys Val Thr His Ala Asp Val
            20                  25                  30
Asp Pro Lys Lys Ala Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr
        35                  40                  45
Tyr Pro Met Leu Arg Leu His Pro Trp His Arg Lys Arg Phe Tyr His
        50                  55                  60
```

FIG.11H

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Arg Phe Gln His Leu Tyr Ala Pro Leu Leu Phe Gly Phe Met Thr Ile
65   70   75   80
Asn Lys Val Ile Thr Gln Asp Val Gly Val Val Leu Ser Lys Arg Leu
            85   90   95
Phe Gln Ile Asp Ala Asn Cys Arg Tyr Ala Ser Lys Ser Tyr Val Ala
        100   105   110
Arg Phe Trp Ile Met Lys Leu Leu Thr Val Leu Tyr Met Val Ala Leu
        115   120   125
Pro Val Tyr Thr Gln Gly Leu Val Asp Gly Leu Lys Leu Phe Phe Ile
    130   135   140
Ala His Phe Ser Cys Gly Glu Leu Leu Ala Thr Met Phe Ile Val Asn
145   150   155   160
His Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ser Val Lys Gly
            165   170   175
Thr Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met His Asp
            180   185   190
Thr Arg Asp Ala Leu Gly Lys Glu Lys Ala Ala Thr Lys His Val Pro
        195   200   205
Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr Ser Val Asn Trp Ser
    210   215   220
Ile Gly Ser Trp Phe Trp Asn His Phe Ser Gly Gly Leu Asn His Gln
225   230   235   240
Ile Glu His His Leu Phe Pro Met Met Met Met
            245   250

FIG.11I

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

FIG.12

```
ccagtgtgct ggaattcagg tactactact acaccatact tacacgaacc tgatcgagat    60
ggagaacggc acccaaaagg tcacccacgc cgacgtcgac cccaagaagg ccgaccagga   120
gagcgacccg gacgtcttca gcacctaccc catgctccgt ctgcacccgt ggcaccgcaa   180
gcgcttctac caccgcttcc agcacctgta cgcgccgctg ctcttcggtt tcatgaccat   240
caacaaggtg atcacccagg atgtgggagt tgtcctcagc aagcgtctgt ttcagatcga   300
tgccaactgc cgttacgcca gcaagtcgta cgttgcgcgc ttctggatca tgaagctgct   360
caccgtcctc tacatggtcg ccctccccgt gtacacccag ggccttgtcg acgggctcaa   420
gctcttcttc atcgcccact tttcgtgcgg cgagctgctg gccaccatgt tcatcgtcaa   480
ccacatcatc gagggcgtct cgtacgcctc caaggactct gtcaagggca ccatggcgcc   540
gccgcgcacg gtgcacggcg tgaccccgat gcatgacacc cgcgacgcgc tcggcaagga   600
gaaggcagcc accaagcacg tgccgctcaa cgactgggcc gcggtccagt gccagacctc   660
ggtcaactgg tcgatcggct cgtggttctg gaaccacttc tccggcgggc tcaaccacca   720
gatcgagcac caccttttcc ccatgatgat gatg                              754
```

FIG.13

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

FIG.14

```
SCORES Initl: 1077  Initn: 1365  Pot: 1371
>>/amd_mnt/home/thurmond/taclone/prta-7.pep      (516 aa)
initn: 1365  initl: 1077  opt: 1371
Smith-Waterman score:1371;   79.1% identity in 249 aa overlap
(5-247:212-460)
```

```
                                              10        20        30
saa.pep                          QCAGTQVLLLHHTYTNLIEMENGTQKVTHADVDP
                                 :| :| || ||||||||||| ||  |||||
prta-7.pep SHGAFSKSRFMNKAAGWTLDMIGASAMTWEMQHVLGHHPYTNLIEMENGLAKVKGADVDP
              190       200       210       220       230       240


                 40        50        60      .70        80        90
saa.pep     KKADQESDPDVFSTYPMLRLHPWHRKRFYHRFQHLYAPLLFGFMTINKVITQDVGVVLSK
            ||:||||||||||||||||||||||||:||||:|||||||||:|||||||||||:|||||||||
prta-7.pep KKVDQESDPDVFSTYPMLRLHPWHRQRFYHKFQHLYAPLIFGFMTINKVISQDVGVVLSK
              250       260       270       280       290       300


                100       110       120       130       140       150
saa.pep     RLFQIDANCRYASKSYVARFWIMKLLTVLYMVALPVYTQGLVDGLKLFFIAHFSCGELLA
            |||||||||||:|   |||||||||||:||||||:| ||  ::|||||:|||:|||:||
prta-7.pep RLFQIDANCRYGSKSYVARFWIMKLLTTLYMVALPMYTQGLVDGLKLFFMAHFTCGEVLA
              310       320       330       340       350       360


                160       170       180       190       200
saa.pep     TMFIVNHIIEGVSYASKDSVKGTMAPPRTVHGVTPMHDTRDALGKEKAA------TKHVP
            ||||||||||||||||||:|||:||||||||||||||: |: ||:  :::     | :|
prta-7.pep TMFIVNHIIEGVSYASKDAVKGVMAPPRTVHGVTPMQVTQKALSAAESTKSDADKTTMIP
              370       380       390       400       410       420


               210       220       230       240       250
saa.pep     LNDWAAVQCQTSVNWSIGSWFWNHFSGGLNHQIEHHLFPMMMM
            |||||||||||||||||::|||||||||||||||||||| ||:
prta-7.pep LNDWAAVQCQTSVNWAVGSWFWNHFSGGLNHQIEHHCFPQNPHTVNVYISGIVKETCEEY
              430       440       450       460       470       480


prta-7.pep GVPYQAEISLFSAYFKMLSHLRTLGNEDLTAWSTX
              490       500       510
```

FIG.15

```
   1 ATGACGGTGG GCGGCGATGA GGTGTACAGC ATGGCGCAGG TGCGCGACCA
  51 CAACACCCCG GACGACGCCT GGTGCGCCAT CCACGGCGAG GTGTACGAGC
 101 TGACCAAGTT CGCCCGCACC CACCCCGGGG GGGACATCAT CTTGCTGGCC
 151 GCCGGCAAGG AGGCCACCAT CCTGTTCGAG ACGTACCACG TGCGCCCCAT
 201 CTCCGACGCG GTCCTGCGCA AGTACCGCAT CGGCAAGCTC GCCGCCGCCG
 251 GCAAGGATGA GCCGGCCAAC GACAGCACCT ACTACAGCTG GGACAGCGAC
 301 TTTTACAAGG TGCTCCGCCA GCGTGTCGTG GCGCGCCTCG AGGAGCGCAA
 351 GATCGCCCGC CGCGGCGGCC CCGAGATCTG GATCAAGGCC GCCATCCTCG
 401 TCAGCGGCTT CTGGTCCATG CTCTACCTCA TGTGCACCCT GGACCCGAAC
 451 CGCGGCGCCA TCCTGGCCGC CATCGCGCTG GGCATCGTCG CCGCCTTCGT
 501 CGGCACGTGC ATTCAGCACG ACGGCAACCA CGGCGCGTTC GCCTTCTCTC
 551 CGTTCATGAA CAAGCTCTCT GGCTGGACGC TCGACATGAT CGGCGCCAGT
 601 GCCATGACCT GGGAGATGCA GCACGTGCTG GGCCACCACC CGTACACCAA
 651 CCTGATCGAG ATGGAGAACG GCACCCAAAA GGTCACCCAC GCCGACGTCG
 701 ACCCCAAGAA GGCCGACCAG GAGAGCGACC CGGACGTCTT CAGCACCTAC
 751 CCCATGCTCC GTCTGCACCC GTGGCACCGC AAGCGCTTCT ACCACCGCTT
 801 CCAGCACCTG TACGCGCCGC TGCTCTTCGG TTTCATGACC ATCAACAAGG
 851 TGATCACCCA GGATGTGGGA GTTGTCCTCA GCAAGCGTCT GTTTCAGATC
 901 GATGCCAACT GCCGTTACGC CAGCAAGTCG TACGTTGCGC GCTTCTGGAT
 951 CATGAAGCTG CTCACCGTCC TCTACATGGT CGCCCTCCCC GTGTACACCC
1001 AGGGCCTTGT CGACGGGCTC AAGCTCTTCT TCATCGCCCA CTTTTCGTGC
1051 GGCGAGCTGC TGGCCACCAT GTTCATCGTC AACCACATCA TCGAGGGCGT
1101 CTCGTACGCC TCCAAGGACT CTGTCAAGGG CACCATGGCG CCGCCGCGCA
1151 CGGTGCACGG CGTGACCCCG ATGCATGACA CCCGCGACGC GCTCGGCAAG
1201 GAGAAGGCAG CCACCAAGCA CGTGCCGCTC AACGACTGGG CCGCGGTCCA
1251 GTGCCAGACC TCGGTCAACT GGTCGATCGG CTCGTGGTTC TGGAACCACT
1301 TCTCCGGCGG GCTCAACCAC CAGATCGAGC ACCACCTCTT CCCCGGCCTC
1351 ACCCACACCA CCTACGTGTA CATTCAGGAT GTGGTGCAGG CGACGTGCGC
1401 CGAGTACGGG GTCCCGTACC AGTCGGAGCA GAGCCTCTTC TCCGCCTACT
1451 TCAAGATGCT CTCCCACCTT CGGGCGCTCG GCAACGAGCC GATGCCCTCG
1501 TGGGAGAAGG ACCACCCCAA GTCCAAGTGA
```

FIG.16

```
  1 MTVGGDEVYS MAQVRDHNTP DDAWCAIHGE VYELTKFART HPGGDIILLA
 51 AGKEATILFE TYHVRPISDA VLRKYRIGKL AAAGKDEPAN DSTYYSWDSD
101 FYKVLRQRVV ARLEERKIAR RGGPEIWIKA AILVSGFWSM LYLMCTLDPN
151 RGAILAAIAL GIVAAFVGTC IQHDGNHGAF AFSPFMNKLS GWTLDMIGAS
201 AMTWEMQHVL GHHPYTNLIE MENGTQKVTH ADVDPKKADQ ESDPDVFSTY
251 PMLRLHPWHR KRFYHRFQHL YAPLLFGFMT INKVITQDVG VVLSKRLFQI
301 DANCRYASKS YVARFWIMKL LTVLYMVALP VYTQGLVDGL KLFFIAHFSC
351 GELLATMFIV NHIIEGVSYA SKDSVKGTMA PPRTVHGVTP MHDTRDALGK
401 EKAATKHVPL NDWAAVQCQT SVNWSIGSWF WNHFSGGLNH QIEHHLFPGL
451 THTTYVYIQD VVQATCAEYG VPYQSEQSLF SAYFKMLSHL RALGNEPMPS
501 WEKDHPKSK
```

FIG.17

```
   1  ATGACGGCCG  GATTTGAAGA  AGTGATCACC  ATGAAGCAGG  TGAAGGACCG
  51  GAATACGCCG  GACGATGCGT  GGTGCGTGGT  GCATGGCAAG  GTGTACGACA
 101  TCACCAAGTT  CAAGAACGCT  CACCCCGGTG  GAGATATAAT  CATGTTGGCG
 151  GCTGGCAAGG  ACGCCACCAT  CCTGTTCGAG  ACTTACCACA  TCCGCGGTGT
 201  GCCCGATGCC  GTGTTGCGCA  AGTATCAGAT  CGGCAAACTT  CCGGACGGAA
 251  AGAACAAAGA  GGGCGGCAAC  GGCCTCGATA  GCGCCTCGTA  CTACTCCTGG
 301  GACAGCGAGT  TTTACCGCGT  CCTTCGCGAG  CGCGTCTTGA  AGCGCCTGAA
 351  CGAGCTCAAG  CTGTCCAGAC  GCGGAGGCTT  CGAGATTTGG  GCCAAGGCTA
 401  TCTTTCTCTT  GACCGGCTTC  TGGTCTTGCC  TCTACCTCAT  GTGCACACTC
 451  AACCCAAATG  GGCTTGCGAT  TCCTGCCGCC  ATGATGTTGG  GAATCTTTGC
 501  TGCCTTCGTA  GGAACCTGCA  TTCAGCACGA  CGGGAATCAC  GGTGCGTTCG
 551  CCCAATCTTC  GTGGCTTAAC  AAGGCCGCTG  GTTGGACTTT  GGACATGATT
 601  GGATCCAGCG  CCATGACCTG  GGAGATGCAG  CACGTGCTTG  GACATCATCC
 651  GTACACCAAC  TTGATTGAAA  TGGAGAATGG  CAATCAAAAG  GTCTCCGGCA
 701  AGCCTGTTGA  CACCAAGACT  GTCGACCAGG  AGAGCGACCC  TGATGTCTTT
 751  AGCACCTACC  CTATGCTTCG  CCTTCACCCT  TGGCACAGCA  AAAAGTGGTA
 801  CCACAAATAC  CAGCACATCT  ATGCACCATT  CATCTTTGGG  TTCATGACCA
 851  TCAACAAGGT  CATTGCACAG  GACGTTGGCG  TTATCACACG  CAAGCGTCTC
 901  TTCCAGATTG  ACGCCAACTG  CCGCTACGCT  TCTCCGACTT  ACGTCGCTCG
 951  CTTCTGGATC  ATGAAGGTTC  TTACCGTTCT  CTACATGGTT  GGCCTGCCAA
1001  TGTACATGCA  AGGTCCATGG  GAGGGTCTCA  AGTTGTTCTT  TATTGCGCAC
1051  TTTACTTGCG  GCGAGCTGCT  GGCCACAATG  TTCATCGTAA  ACCACATCAT
1101  CGAGGGTGTC  AGCTACGCAA  GCAAAGATGC  CATCAAGGGC  GAGATGGCTC
1151  CACCGAAAAC  GGTCCGCGGT  GTCACCCCAA  TGCACGAGAC  GCAAAAGGTT
1201  CTCGACCAGC  GCGAGAAAGA  CATGGACGAA  ACTTCTAAGA  AGAGCCGCAT
1251  CCCTCTCAAC  GACTGGGCCG  CTGTACAGTG  CCAGACCACC  GTGAACTGGG
1301  CTATCGGTTC  TTGGTTCTGG  AACCACTTTT  CCGGGGGCCT  CAATCATCAG
1351  ATTGAGCATC  ATCTGTTCCC  CGGCTTGACT  CACACCACCT  ATGTTCACTT
1401  TCACGATGTG  GTCAAAGATA  CTTGCGCTGA  GTACGGGGTT  CCATACCAGC
1451  ACGAGGAGAG  TCTATACACT  GCCTACTTTA  AGATGTTGAA  TCATCTCAAG
1501  ACCCTAGGCA  ACGAGCCAAT  GCCTGCCTGG  GACAAGAACT  AA
```

FIG.18

```
  1   MTAGFEEVIT MKQVKDRNTP DDAWCVVHGK VYDITKFKNA HPGGDIIMLA
 51   AGKDATILFE TYHIRGVPDA VLRKYQIGKL PDGKNKEGGN GLDSASYYSW
101   DSEFYRVLRE RVLKRLNELK LSRRGGFEIW AKAIFLLTGF WSCLYLMCTL
151   NPNGLAIPAA MMLGIFAAFV GTCIQHDGNH GAFAQSSWLN KAAGWTLDMI
201   GSSAMTWEMQ HVLGHHPYTN LIEMENGNQK VSGKPVDTKT VDQESDPDVF
251   STYPMLRLHP WHSKKWYHKY QHIYAPFIFG FMTINKVIAQ DVGVITRKRL
301   FQIDANCRYA SPTYVARFWI MKVLTVLYMV GLPMYMQGPW EGLKLFFIAH
351   FTCGELLATM FIVNHIIEGV SYASKDAIKG EMAPPKTVRG VTPMHETQKV
401   LDQREKDMDE TSKKSRIPLN DWAAVQCQTT VNWAIGSWFW NHFSGGLNHQ
451   IEHHLFPGLT HTTYVHFHDV VKDTCAEYGV PYQHEESLYT AYFKMLNHLK
501   TLGNEPMPAW DKN
```

FIG.19

DNA sequence of the Delta 4 desaturase gene (pRIG6) from
Isochrysis galbana (CCMP 1323)

```
   1  ATGTGCAACG  CGGCGCAGGT  CGAGACGCAG  GCCTTGCGCG  CCAAGGAGGC
  51  GGCAAAACCG  ACGTGGACGA  AGATTCATGG  GCGCACAGTC  GACGTGGAGA
 101  CGTTCCGCCA  CCCAGGCGGC  AACATCCTCG  ATTTGTTCCT  GGGCATGGA
 151  GCCACAACTG  CCTTTGAGAC  GTTCCACGGT  CACCACAAGG  GAGCATGGAA
 201  GATGCTCAAG  ACGCTGCCCG  AGAAGGAGGT  CGCCGCCGCC  GACATTCCCG
 251  CGCAGAAGGA  GGAGCACGTG  GCCGAGATGA  CACGCCTCAT  GGCCTCATGG
 301  CGCGAGCGCG  GGCTGTTCAA  GCCGCGTCCC  GTCGCCTCAT  CCATCTATGG
 351  CCTGTGCGTG  ATCTTCGCCA  TCGCGGCATC  GGTCGCGTGC  GCTCCGTACG
 401  CGCCAGTGCT  GGCTGGCATC  GCGGTGGGCA  CCTGCTGGGC  TCAGTGCGGC
 451  TTCTTGCAGC  ACATGGGCGG  CCACCGGGAG  TGGGGGCGCA  CTTGGTCGTT
 501  TGCGTTTCAG  CATCTGTTTG  AAGGCCTGCT  CAAGGGCGGC  TCGGCCTCGT
 551  GGTGGCGCAA  CCGCCACAAC  AAGCACCATG  CCAAGACCAA  CGTGCTCGGC
 601  GAGGACGGCG  ACCTGCGCAC  CACACCCTTC  TTCGCATGGG  ACCCTACTCT
 651  GGCCAAGAAA  GTGCCCGACT  GGTCTCTGCG  CACGCAAGCC  TTCACCTTTC
 701  TGCCAGCACT  GGGAGCTTAC  GTCTTCGTCT  TTGCCTTCAC  GGTACGCAAG
 751  TACAGTGTGG  TGAAGCGTCT  CTGGCACGAG  GTCGCCCTGA  TGGTGGCCCA
 801  CTACGCTCTC  TTTTCCTGGG  CGCTCAGCGC  CGCCGGCGCC  TCCCTCAGCT
 851  CCGGCCTCAC  CTTCTATTGC  ACCGGGTACG  CCTGGCAGGG  CATCTACCTC
 901  GGCTTCTTCT  TCGGCCTATC  GCACTTTGCG  GTGGAGCGCG  TGCCGTCGAC
 951  CGCCACCTGG  CTCGAGTCGA  CGATGATGGG  CACCGTTGAC  TGGGGCGGCT
1001  CCTCCGCCTT  CTGCGGCTAC  CTCTCCGGCT  TCCTCAATAT  CCAGATCGAG
1051  CACCACATGG  CTCCACAAAT  GCCAATGGAG  AACCTGCGCC  AGATCCGGGC
1101  CGACTGCAAG  GCCGCGGCCC  ACAAGTTCGG  GCTGCCGTAC  CGCGAGCTGA
1151  CATTCGTCGC  GGCGACCAAG  CTCATGATGA  GCGGCCTCTA  CCGGACCGGC
1201  AAGGACGAGC  TCAAGCTGCG  CGCGGACCGC  CGCAAGTTCA  CGAGGGCACA
1251  GGCGTACATG  GGCGCCGCCA  GCGCTTTGGT  CGACACGCTC  AAGGCGGACT
1301  AA
```

FIG.20

Amino acid sequence of the Delta 4 desaturase gene (pRIG6)
from _Isochrysis galbana_ (CCMP 1323)

```
  1  MCNAAQVETQ ALRAKEAAKP TWTKIHGRTV DVETFRHPGG NILDLFLGMD

 51  ATTAFETFHG HHKGAWKMLK TLPEKEVAAA DIPAQKEEHV AEMTRLMASW

101  RERGLFKPRP VASSIYGLCV IFAIAASVAC APYAPVLAGI AVGTCWAQCG

151  FLQHMGGHRE WGRTWSFAFQ HLFEGLLKGG SASWWRNRHN KHHAKTNVLG

201  EDGDLRTTPF FAWDPTLAKK VPDWSLRTQA FTFLPALGAY VFVFAFTVRK

251  YSVVKRLWHE VALMVAHYAL FSWALSAAGA SLSSGLTFYC TGYAWQGIYL

301  GFFFGLSHFA VERVPSTATW LESTMMGTVD WGGSSAFCGY LSGFLNIQIE

351  HHMAPQMPME NLRQIRADCK AAAHKFGLPY RELTFVAATK LMMSGLYRTG

401  KDELKLRADR RKFTRAQAYM GAASALVDTL KAD*
```

FIG.21

Comparative analysis of the Delta 4 desaturase from
I. galbana (CCMP 1323) (pRIG6.pep) and the Delta 4 desaturase
from T. aureum (ATCC 34304)(pRTA7.pep)

Quality: 68   Length: 554
Ratio: 0.157   Gaps: 22
Percent Similarity: 38.384   Percent Identity: 30.808

Match display thresholds for the alignment(s):
                    | = IDENTITY
                    : = 2
                    . = 1

pRIG6.pepx prta7.pep

```
    .         .         .         .         .
  1 ..MCNAAQVETQALRAKEAAKPTWTKIHGRTVDVETFR..HPGGNILDLF 46
       .   |     .|      |  |||   |:  |   ||||.|: |
  1 MTVGFDETVTMDTVRNHNMPDDAWCAIHGTVYDITKFSKVHPGGDIIMLA 50

         .         .         .         .         .
 47 LGMDATTAFETFH..GHHKGAWKMLKT..LPE.KEVAAADIPAQKE.... 87
     | :||  |||:| |         :  |    ||: |.   ..|   :
 51 AGKEATILFETYHIKGVPDAVLRKYKVGKLPQGKKGETSHMPTGLDSASY 100

         .         .         .         .         .
 88 ...........EHVAE.......MTRLMASWRERGLFKPRPVASSIYGL 118
              | ||.       || |      : :|      |:| :
101 YSWDSEFYRVLRERVAKKLAEPGLMQRARMELWAKAIFLLAGFWGSLYAM 150

         .         .         .         .         .
119 CVIFAIAASVACAPYAPVLAGIAVGTCWAQCGFLQHMGGHREWGRTWSFA 168
     ||:       ..   |   | |  ||||      :|| |   . :.
151 CVLDPHGGAMVAAVTLGVFAAF.VGTC......IQHDGSHGAFSKS...R 190

         .         .         .         .         .
169 FQHLFEGL...LKGGSASWWRNRH.NKHHAKTNVLGEDGDLRTTPFFAWD 214
     | .   |   : | || |  .|  || ||.:  :   |          |
191 FMNKAAGWTLDMIGASAMTWEMQHVLGHHPYTNLIEMENGLAKVKGADVD 240

         .         .         .         .         .
215 PTLAKKVPDWSLRTQAFTFLPALGAYVF...............VFAF.T 247
     |  ||| |          |. | |  : .            :| | |
241 P...KKV.DQESDPDVFSTYPMLRLHPWHRQRFYHKFQHLYAPLIFGFMT 286

         .         .         .         .         .
248 VRK.....YSVV..KRL...........WHEVA..LMVAHYALFSWALS 276
     : |      ||  |||            |.    :|    |: ||
287 INKVISQDVGVVLRKRLFQIDANCRYGSPWNVARFWIMKLLTTLYMVALP 336

         .         .         .         .         .
277 AAGASLSSGLTFYCTGYAWQGIYLGFFFGLSHF................ 309
       .||  :  :  |  | |  |..|
337 MYMQGPAQGLKLFFMAHFTCGEVLATMFIVNHIIEGVSYASKDAVKGVMA 386
```

FIG.22A

```
310  ...................AVERVPSTA.........TWLESTMMGTVDW 331
                        | |   | |           |      .|.|
387  PPRTVHGVTPMQVTQKALSAAESTKSDADKTTMIPLNDWAAVQCQTSVNW 436

332  GGSSAFCGYLSGFLNIQIEHHMAPQMPMENLRQIRADCKAAAHKFGLPYR 381
     | |  :  || || |||||  || |       |     |   .:|.||.
437  AVGSWFWNHFSGGLNHQIEHHCFPQNPHTVNVYISGIVKETCEEYGVPYQ 486

382  .ELTFVAATKLMMSGLYRTGKDELKLRADRRKFTRAQAYMGAASALVDTL 430
     |:.  .|    |:| |   | ::|  .
487  AEISLFSAYFKMLSHLRTLGNEDLTAWST*.................... 516
```

FIG.22B

SEQUENCE LISTING

<110> Abbott Laboratories
      Mukerji, Pradip
      Thurmond, Jennifer M.
      Huang, Yung-Sheng
      Das, Tapas
      Leonard, Amanda E.
      Pereira, Suzette L.

<120> DELTA 4-DESATURASE GENES AND USES
   THEREOF

<130> 6804.US.P1

<140> Not Yet Assigned
<141> 2002-04-11

<150> US 09/849,199
<151> 2001-05-04

<160> 73

<170> FastSEQ for Windows Version 4.0

<210> 1
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<212> DNA
<213> Artificial Sequence

<220>
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<222> (3)...(3)
<223> b = g or c or t/u at position 3

<221> misc_feature
<222> (6)...(6)
<223> y = t/u or c at position 6

<221> misc_feature
<222> (9)...(9)
<223> y = t/u or c at position 9

<221> misc_feature
<222> (12)...(12)
<223> b = g or c or t/u at position 12

<221> misc_feature
<222> (18)...(18)
<223> r = g or a at position 18

<221> misc_feature
<222> (24)...(24)
<223> b = g or c or t/u at position 24

```
<221> misc_feature
<222> (30)...(30)
<223> y = t/u or c at position 30

<221> misc_feature
<222> (33)...(33)
<223> y = t/u or c at position 33

<221> misc_feature
<222> (36)...(36)
<223> b = g or c or t/u at position 36

<221> misc_feature
<222> (39)...(39)
<223> h = a or c or t/u at position 39

<221> misc_feature
<222> (42)...(42)
<223> h = a or c or t/u at position 42

<400> 1
gtbtaygayg tbaccgartg ggtbaagcgy cayccbgghg gh                        42

<210> 2
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Forward Primer RO835

<221> misc_feature
<222> (3)...(3)
<223> h = a or c or t/u at position 3

<221> misc_feature
<222> (6)...(6)
<223> y = t/u or c at position 6

<221> misc_feature
<222> (12)...(12)
<223> y = t/u or c at position 12

<221> misc_feature
<222> (27)...(27)
<223> y = t/u or c at position 27

<221> misc_feature
<222> (33)...(33)
<223> y = t/u or c at position 33

<221> misc_feature
<222> (39)...(39)
<223> b = g or c or t/u at position 39

<221> misc_feature
```

```
<222> (42)...(42)
<223> y = t/u or c at position 42

<221> misc_feature
<222> (45)...(45)
<223> y = t/u or c at position 45

<400> 2
gghgcytccg cyaactggtg gaagcaycag cayaacgtbc aycay            45

<210> 3
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse Primer RO836

<221> misc_feature
<222> (1)...(1)
<223> r = g or a at position 1

<221> misc_feature
<222> (4)...(4)
<223> r = g or a at position 4

<221> misc_feature
<222> (7)...(7)
<223> v = a or g or c at position 7

<221> misc_feature
<222> (13)...(13)
<223> r = g or a at position 13

<221> misc_feature
<222> (19)...(19)
<223> r = g or a at position 19

<221> misc_feature
<222> (34)...(34)
<223> r = g or a at position 34

<221> misc_feature
<222> (40)...(40)
<223> r = g or a at position 40

<221> misc_feature
<222> (43)...(43)
<223> d = a or g or t/u at position 43

<400> 3
rtgrtgvacg ttrtgctgrt gcttccacca gttrgcggar gcdcc           45

<210> 4
<211> 36
<212> DNA
<213> Artificial Sequence
```

<220>
<223> Reverse Primer RO838

<221> misc_feature
<222> (6)...(6)
<223> r = g or a at position 6

<221> misc_feature
<222> (12)...(12)
<223> r = g or a at position 12

<221> misc_feature
<222> (15)...(15)
<223> y = t/u or c at position 15

<221> misc_feature
<222> (18)...(18)
<223> r = g or a at position 18

<221> misc_difference
<222> (21)...(21)
<223> r = g or a at position 21

<221> misc_feature
<222> (24)...(24)
<223> s = g or c at position 24

<221> misc_feature
<222> (27)...(27)
<223> r = g or a at position 27

<221> misc_feature
<222> (30)...(30)
<223> v = a or g or c at position 30

<400> 4
ttgatrgtct arctygtrgt rgasaarggv tggtac   36

<210> 5
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO753

<221> misc_feature
<222> (10)...(10)
<223> n = a or g or c or t/u, unknown or other at
      position 10

<221> misc_feature
<222> (13)...(13)
<223> r = g or a at position 13

<221> misc_feature

```
<222>  (16)...(16)
<223>  n = a or g or c or t/u, unknown or other at
       position 16

<221>  misc_feature
<222>  (18)...(19)
<223>  r = g or a at positions 18-19

<221>  misc_feature
<222>  (22)...(22)
<223>  r = g or a at position 22

<400>  5
catcatcatn ggraanarrt grtg                                    24

<210>  6
<211>  30
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Primer RO754

<221>  misc_feature
<222>  (15)...(15)
<223>  y = t/u or c at position 15

<221>  misc_feature
<222>  (18)...(18)
<223>  y = t/u or c at position 18

<221>  misc_feature
<222>  (21)...(21)
<223>  n = a or g or c or t/u, unknown, or other at
       position 21

<221>  misc_feature
<222>  (24)...(24)
<223>  y = t/u or c at position 24

<221>  misc_feature
<222>  (27)...(27)
<223>  n = a or g or c or t/u, unknown, or other at
       position 27

<221>  misc_feature
<222>  (30)...(30)
<223>  y = t/u or c at position 30

<400>  6
ctactactac tacaycayac ntayacnaay                              30

<210>  7
<211>  31
<212>  DNA
<213>  Artificial Sequence
```

```
<220>
<223> Primer RO898

<400> 7
cccagtcacg acgttgtaaa acgacggcca g                                    31

<210> 8
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO930

<400> 8
gacgattaac aaggtgattt cccaggatgt c                                    31

<210> 9
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO973

<400> 9
gactaactcg agtcacgtgg accaggccgt gaggtcct                             38

<210> 10
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO974

<400> 10
gactaactcg agttgacgag gtttgtatgt tcggcggttt gcttg                     45

<210> 11
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO899

<400> 11
agcggataac aatttcacac aggaaacagc                                      30

<210> 12
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO1004
```

```
<400> 12
tggctaccgt cgtgctggat gcaagttccg                                    30


<210> 13
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO1046

<400> 13
cgcatggaat tcatgacggt cgggtttgac gaaacggtg                          39


<210> 14
<211> 1548
<212> DNA
<213> Thraustochytrium aureum

<400> 14
atgacggtcg ggtttgacga aacggtgact atggacacgg tccgcaacca caacatgccg    60
gacgacgcct ggtgcgcgat ccacggcacc gtgtacgaca tcaccaagtt cagcaaggtg   120
caccccggcg gggacatcat catgctggcc gctggcaagg aggccaccat cctgttcgag   180
acgtaccaca tcaagggcgt cccggacgcg gtgctgcgca agtacaaggt cggcaagctc   240
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gacatgatcg gcgcgagcgc gatgacctgg gagatgcagc acgttcttgg ccaccacccg   660
tacaccaacc tcatcgagat ggagaacggt ttggccaagg tcaagggcgc cgacgtcgac   720
ccgaagaagg tcgaccagga gagcgacccg gacgtcttca gtacgtaccc gatgcttcgc   780
ctgcaccgt ggcaccgcca gcggttttac cacaagttcc agcacctgta cgccccgttt    840
atctttgggt ttatgacgat taacaaggtg atttcccagg atgtcggggt tgtgctgcgc   900
aagcgcctgt tccagatcga cgccaactgc cggtatggca gcccctggta cgtggcccgc   960
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cccctcaacg actgggccgc tgtgcagtgc cagacctctg tgaactgggc tgtcgggtcg  1320
tggttttgga accactttc gggcggcctc aaccaccaga ttgagcacca ctgcttcccc   1380
caaaaccccc acacggtcaa cgtctacatc tcgggcatcg tcaaggagac ctgcgaagaa  1440
tacggcgtgc cgtaccaggc tgagatcagc ctcttctctg cctatttcaa gatgctgtcg  1500
cacctccgca cgctcggcaa cgaggacctc acggccaggt ccacgtga               1548


<210> 15
<211> 1548
<212> DNA
<213> Thraustochytrium aureum

<400> 15
atgacggtcg ggtttgacga aacggtgact atggacacgg tccgcaacca caacatgccg    60
gacgacgcct ggtgcgcgat ccacggcacc gtgtacgaca tcaccaagtt cagcaaggtg   120
caccccggcg gggacatcat catgctggcc gctggcaagg aggccaccat cctgttcgag   180
```

```
acgtaccaca tcaagggcgt tccggacgcg gtgctgcgca agtacaaggt cggcaagctc    240
ccccagggca agaagggcga aacgagccac atgcccaccg ggctcgactc ggccttctac    300
tactcgtggg acagcgagtt ttacagggtg ctccgcgagc gcgtcgccaa gaagctggcc    360
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gtagccgccg ttacgctcgg cgtgttcgct gcctttgtcg gaacttgcat ccagcacgac    540
ggcagccacg gcgccttctc caagtcgcga ttcatgaaca aggcggcggg ctggaccctc    600  .
gacatgatcg gcgcgagcgc gatgacctgg gagatgcagc acgttcttgg ccaccacccg    660
tacaccaacc tcatcgagat ggagaacggt ttggccaagg tcaagggcgc cgacgtcgac    720
ccgaagaagg tcgaccagga gagcgacccg gacgtcttca gtacgtaccc gatgcttcgc    780
ctgcaccgt ggcaccgcca gcggttttac cacaagttcc agcacctgta cgccccgttt    840
atctttgggt ttatgacgat taacaaggtg atttcccagg atgtcggggt tgtgctgcgc    900
aagcgcctgt tccagatcga cgccaactgc cggtatggca gccccctggta cgtggcccgc    960
ttctggatca tgaagctcct caccacgctc tacatggtgg cgcttcccat gtacatgcag .  1020
gggcctgctc agggcttgaa gctttttcttc atggcccact tcacctgcgg agaggtcctc   1080
gccaccatgt ttattgtcaa ccacatcatc gagggcgtca gctacgcttc caaggacgcg   1140
gtcaagggcg tcatggctcc gccgcgcact gtgcacggtg tcaccccgat gcaggtgacg   1200
caaaaggcgc tcagtgcggc cgagtcgacc aagtcggacg ccgacaagac gaccatgatc   1260
cccctcaacg actgggccgc tgtgcagtgc cagacctctg tgaactgggc tgtcgggtcg   1320
tggtttttgga accactttttc gggcggcctc aaccaccaga ttgagcacca ctgcttcccc   1380
caaaacccccc acacggtcaa cgtctacatc tcaggcatcg tcaaggagac ctgcgaagaa   1440
tacggcgtgc cgtaccaggc tgagatcagc ctcttctctg cctatttcaa gatgctgtcg   1500
cacctccgca cgctcggcaa cgaggacctc acggcctggt ccacgtga              1548
```

```
<210> 16
<211> 1548
<212> DNA
<213> Thraustochytrium aureum

<400> 16
atgacggtcg ggtttgacga aacggtgact atggacacgg tccgcaacca caacatgccg     60
gacgacgcct ggtgcgcgat ccacggcacc gtgtacgaca tcaccaagtt cagcaaggtg    120
caccccggcg gggacatcat catgctggcc gctggcaagg aggccaccat cctgttcgag    180
acctaccaca tcaagggcgt cccggacgcg gtgctgcgca agtacaaggt cggcaagctc    240
ccccagggca agaagggcga aacgagccac atgcccaccg ggctcgactc ggcctcctac    300
tactcgtggg acagcgagtt ttacagggtg ctccgcgagc gcgtcgccaa gaagctggcc    360
gagcccggcc tcatgcagcg cgcgcgcatg gagctctggg ccaaggcgat cttcctcctg    420
gcaggtttct ggggctccct ttacgccatg tgcgtgctag acccgcacgg cggtgccatg    480
gtagccgccg ttacgctcgg cgtgttcgct gcctttgtcg gaacttgcat ccagcacgac    540
ggcagccacg gcgccttctc caagtcgcga ttcatgaaca aggcggcggg ctggaccctc    600
gacatgatcg gcgcgagcgc gatgacctgg gagatgcagc acgttcttgg tcaccacccg    660
tacaccaacc tcatcgagat ggagaacggt ttggccaagg tcaagggcgc cgacgtcgac    720
ccgaagaagg tcgaccagga gagcgacccg gacgtcttca gtacgtaccc gatgcttcgc    780
ctgcaccgt ggcaccgcca gcggttttac cacaagttcc agcacctgta cgccccgctt    840
atctttgggt ttatgacgat taacaaggtg atttcccagg atgtcggggt tgtgctgcgc    900
aagcgcctgt tccagatcga cgccaactgc cggtatggca gccccctggaa cgtggcccgc    960
ttctggatca tgaagctcct caccacgctc tacatggtgg cgcttcccat gtacatgcag   1020
gggcctgctc agggcttgaa gctttttcttc atggcccact tcacctgcgg agaggtcctc   1080
gccaccatgt ttattgtcaa ccacatcatc gagggcgtca gctacgcttc caaggacgcg   1140
gtcaagggcg tcatggctcc gccgcgcact gtgcacggtg tcaccccgat gcaggtgacg   1200
caaaaggcgc tcagtgcggc cgagtcgacc aagtcggacg ccgacaagac gaccatgatc   1260
cccctcaacg actgggccgc tgtgcagtgc cagacctctg tgaactgggc tgtcgggtcg   1320
tggtttttgga accactttttc gggcggccta aaccaccaga ttgagcacca ctgcttcccc   1380
caaaacccccc acacggtcaa cgtctacatc tcgggcatcg tcaaggagac ctgcgaagaa   1440
tacggcgtgc cgtaccaggc tgagatcagc ctcttctctg cctatttcaa gatgctgtcg   1500
cacctccgca cgctcggcaa cgaggacctc acggcctggt ccacgtga              1548
```

```
<210> 17
<211> 1548
<212> DNA
<213> Thraustochytrium aureum

<400> 17
atgacggtcg ggtttgacga aacggtgact atggacacgg tccgcaacca caacatgccg      60
gacgacgcct ggtgcgcgat ccacggcacc gtgtacgaca tcaccaagtt cagcaaggtg     120
cacccggcg gggacatcat catgctggcc gctggcaagg aggccaccat cctgttcgag      180
acctaccaca tcaagggcgt cccggacgcg gtgctgcgca agtacaaggt cggcaagctc     240
ccccagggca agaagggcga aacgagccac atgcccaccg ggctcgactc ggcctcctac     300
tactcgtggg acagcgagtt ttacagggtg ctccgcgagc gcgtcgccaa gaagctggcc     360
gagcccggcc tcatgcagcg cgcgcgcatg gagctctggg ccaaggcgat cttcctcctg     420
gcaggtttct ggggctccct ttacgccatg tgcgtgctag acccgcacgg cggtgccatg .   480
gtagccgccg ttacgctcgg cgtgttcgct gcctttgtcg aacttgcat ccagcacgac      540
ggcagccacg gcgccttctc caagtcgcga ttcatgaaca aggcggcggg ctggaccctc     600
gacatgatcg gcgcgagtgc gatgacctgg gagatgcagc acgttcttgg ccaccacccg     660
tacaccaacc tcatcgagat ggagaacggt ttggccaagg tcaagggcgc cgacgtcgac     720
ccgaagaagg tcgaccagga gagcgacccg gacgtcttca gtacgtaccc gatgcttcgc     780
ctgcaccgt ggcaccgcca gcggttttac cacaagttcc agcacctgta cgccccgttt      840
atctttgggt ctatgacgat taacaaggtg atttcccagg atgtcggggt tgtgctgcgc     900
aagcgcctgt ccagatcga cgccaactgc cggtatggca gccctggta cgtggcccgc       960
ttctggatca tgaagctcct caccacgctc tacatggtgg cgcttccat gtacatgcag     1020
gggcctgctc agggcttgaa gcttttcttc atggcccact tcacctgcgg agaggtcctc    1080
gccaccatgt ttattgtcaa ccacatcatc gagggcgtca gctacgcttc caaggacgcg    1140
gtcaagggcg tcatggctcc gccgcgcact gtgcacggtg tcaccccgat gcaggtgacg    1200
caaaaggcgc tcagtgcggc cgagtcggcc aagtcggacg ccgacaagac gaccatgatc    1260
cccctcaacg actgggccgc tgtgcagtgc cagacctctg tgaactgggc tgtcgggtcg    1320
tggttttgga accactttc gggcggcctc aaccaccaga ttgagcacca ctgcttcccc    1380
caaaaccccc acacggtcaa cgtctacatc tcgggcatcg tcaaggagac ctgcgaagaa    1440
tacggcgtgc cgtaccaggc tgagatcagc ctcttctctg cctatttcaa gatgctgtcg    1500
cacctccgca cgctcggcaa cgaggacctc acggcctggt ccacgtga               1548

<210> 18
<211> 515
<212> PRT
<213> Thraustochytrium aureum

<400> 18
Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn
1               5                   10                  15
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
            20                  25                  30
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
            35                  40                  45
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
        50                  55                  60
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu
65                  70                  75                  80
Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp
                85                  90                  95
Ser Ala Ser Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg
            100                 105                 110
Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala
            115                 120                 125
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
        130                 135                 140
```

```
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met
145             150                 155                 160
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys
                165                 170                 175
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
            180                 185                 190
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
        195                 200                 205
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu
    210                 215                 220
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
225                 230                 235                 240
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
                245                 250                 255
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
            260                 265                 270
Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Phe Met Thr Ile Asn
        275                 280                 285
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
    290                 295                 300
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg
305                 310                 315                 320
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Thr Val Ala Leu Pro
                325                 330                 335
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
            340                 345                 350
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
        355                 360                 365
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
    370                 375                 380
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385                 390                 395                 400
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys
                405                 410                 415
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
            420                 425                 430
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
        435                 440                 445
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
    450                 455                 460
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
465                 470                 475                 480
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
                485                 490                 495
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
                500                 505                 510
Arg Ser Thr
            515
```

```
<210> 19
<211> 515
<212> PRT
<213> Thraustochytrium aureum

<400> 19
Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn
  1             5                 10                  15
```

```
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
            20              25              30
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
        35              40          .       45
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
    50              ,   55              60
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu
65              70              75                      80
Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp
                85              90                      95
Ser Ala Phe Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg
            100             105             110
Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala
        115             120             125
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
    130             135             140
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met
145             150             155                     160
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys
            165             170             175
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
            180             185             190
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
            195             200             205
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu
    210             215             220
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
225             230             235             240
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
            245             250             255
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
            260             265             270
Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Phe Met Thr Ile Asn
    275             280             285
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
    290             295    /        300
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg
305             310             315                     320
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro
            325             330             335
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
            340             345             350
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
            355             360             365
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
    370             375             380
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385             390             395                     400
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys
            405             410                     415
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
            420             425             430
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
            435             440             445
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
450             455             460
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
```

```
465                      470                      475                      480
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
                485                      490                      495.
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
            500                      505                      510
Trp Ser Thr
        515
```

<210> 20
<211> 515
<212> PRT
<213> Thraustochytrium aureum

<400> 20
```
Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn
 1   ,           5                  10                  15
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
            20                  25                  30
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
            35                  40                  45
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
        50                  55                  60
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu
65                  70                  75                  80
Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp
                85                  90                  95
Ser Ala Ser Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg
            100                 105                 110
Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala
            115                 120                 125
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
        130                 135                 140
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met
145                 150                 155                 160
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys
            165                 170                 175
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
            180                 185                 190
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
            195                 200                 205
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu
        210                 215                 220
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
225                 230                 235                 240
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
            245                 250                 255
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
            260                 265                 270
Phe Gln His Leu Tyr Ala Pro Leu Ile Phe Gly Phe Met Thr Ile Asn
        275                 280                 285
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
        290                 295                 300
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Asn Val Ala Arg
305                 310                 315                 320
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro
            325                 330                 335
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
```

```
              340                    345                    350
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
          355                    360                    365
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
      370                    375                    380
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385                    390                    395                    400
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys
              405                    410                    415
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
          420                    425                    430
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
          435                    440                    445
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
      450                    455                    460
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
465                    470                    475                    480
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
              485                    490                    495
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
          500                    505                    510
Trp Ser Thr
          515
```

<210> 21
<211> 515
<212> PRT
<213> Thraustochytrium aureum

<400> 21

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

```
            210                     215                     220
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
225                     230                     235                     240
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
                245                     250                     255
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
            260                     265                     270
Phe Gln His Leu Tyr Ala Pro Phe Ile Phe Gly Ser Met Thr Ile Asn
            275                     280                     285
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
            290                     295                     300
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Tyr Val Ala Arg
305                     310                     315                     320
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro
                325                     330                     335
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
            340                     345                     350
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
            355                     360                     365
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
    370                     375              .       380
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385                     390                     395                     400
Gln Lys Ala Leu Ser Ala Ala Glu Ser Ala Lys Ser Asp Ala Asp Lys
                405                     410                     415
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
              420                     425                     430
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
            435                     440                     445
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
    450                     455                     460
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
465                     470                     475                     480
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
                485                     490                     495
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
            500                     505                     510
Trp Ser Thr
            515
```

```
<210> 22
<211> 879
<212> DNA
<213> Mus musculus
```

```
<400> 22
atggagcagc tgaaggcctt tgataatgaa gtcaatgctt tcttggacaa catgtttgga    60
ccacgagatt ctcgagttcg cgggtggttc ctgctggact cttaccttcc caccttcatc   120
ctcaccatca cgtacctgct ctcgatatgg ctgggtaaca agtacatgaa gaacaggcct   180
gctctgtctc tcaggggcat cctcaccttg tataacctcg caatcacact tctttctgcg   240
tatatgctgg tggagctcat cctctccagc tgggaaggag gttacaactt gcagtgtcag   300
aatctcgaca gtgcaggaga aggtgatgtc cgggtagcca aggtcttgtg tgtggtactac   360
ttctccaaac tagtggagtt cctggacacg attttctttg ttctacgaaa aaagaccaat   420
cagatcacct tccttcatgt ctatcaccac gcgtccatgt caacatctg gtggtgtgtt   480
ttgaactgga taccttgtgg tcaaagcttc tttggaccca ccctgaacag ctttatccac   540
attctcatgt actcctacta cggcctgtct gtgttcccgt ccatgcacaa gtacctttgg   600
tggaagaagt acctcacaca ggctcagctg gtgcagttcg tactcaccat cacgcacacg   660
```

```
ctgagtgccg tggtgaagcc ctgtggcttc ccctttggct gtctcatctt ccagtcttcc    720
tatatgatga cgctggtcat cctgttctta aacttctata ttcagacata ccggaaaaag    780
ccagtgaaga aagagctgca agagaaagaa gtgaagaatg gtttccccaa agcccactta    840
attgtggcta atggcatgac ggacaagaag gctcaataa                           879
```

<210> 23
<211> 292
<212> PRT
<213> Mus musculus

<400> 23

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

<210> 24
<211> 754
<212> DNA
<213> Schizochytrium aggregatum

<400> 24

```
ccagtgtgct ggaattcagg tactactact acaccatact tacacgaacc tgatcgagat    60
```

```
ggagaacggc acccaaaagg tcacccacgc cgacgtcgac cccaagaagg ccgaccagga   120
gagcgacccg gacgtcttca gcacctaccc catgctccgt ctgcacccgt ggcaccgcaa   180
gcgcttctac caccgcttcc agcacctgta cgcgccgctg ctcttcggtt tcatgaccat   240
caacaaggtg atcacccagg atgtgggagt tgtcctcagc aagcgtctgt ttcagatcga   300
tgccaactgc cgttacgcca gcaagtcgta cgttgcgcgc ttctggatca tgaagctgct   360
caccgtcctc tacatggtcg ccctccccgt gtacacccag ggccttgtcg acgggctcaa   420
gctcttcttc atcgcccact tttcgtgcgg cgagctgctg gccaccatgt tcatcgtcaa   480
ccacatcatc gagggcgtct cgtacgcctc caaggactct gtcaagggca ccatggcgcc   540
gccgcgcacg gtgcacggcg tgaccccgat gcatgacacc cgcgacgcgc tcggcaagga   600
gaaggcagcc accaagcacg tgccgctcaa cgactgggcc gcggtccagt gccagacctc   660
ggtcaactgg tcgatcggct cgtggttctg gaaccacttc tccggcgggc tcaaccacca   720
gatcgagcac cacctttttcc ccatgatgat gatg                              754
```

<210> 25
<211> 251
<212> PRT
<213> Schizochytrium aggregatum

<400> 25

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

<210> 26
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO1240

<400> 26
ccctcgatga tgtggttgac gatgaac   27

<210> 27
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' Nested Primer RO1239

<400> 27
cggagcatgg ggtaggtgct gaagac   26

<210> 28
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO1236

<400> 28
ccaactgccg ttacgccagc aagt   24

<210> 29
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> 3' Nested Primer RO1237

<400> 29
caagctcttc ttcatcgccc acttttcg   28

<210> 30
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO1240 and GeneRacer 5' Primer

<400> 30
cgactggagc acgaggacac tga   23

<210> 31
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO1236 and GeneRacer 3' Primer

```
<400> 31
gctgtcaacg atacgctacg taacg                                              25

<210> 32
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Nested Primer RO1239 and GeneRacer Nested 5'
      Primer

<400> 32
ggacactgac atggactgaa ggagta                                            26

<210> 33
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Nested Primer RO1237 and GeneRacer Nested 3'
      Primer

<400> 33
cgctacgtaa cggcatgaca gtg                                                23

<210> 34
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO1241

<400> 34
gatatcgaat tcatgacggt gggcggcgat gagg                                    34

<210> 35
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO1242

<400> 35
gtacttaagc tttcacttgg acttggggtg gtcc                                    34

<210> 36
<211> 1530
<212> DNA
<213> Schizochytrium aggregatum

<400> 36
atgacggtgg gcggcgatga ggtgtacagc atggcgcagg tgcgcgacca caacaccccg      60
```

```
gacgacgcct ggtgcgccat ccacggcgag gtgtacgagc tgaccaagtt cgcccgcacc    120
cacccccgggg gggacatcat cttgctggcc gccggcaagg aggccaccat cctgttcgag    180
acgtaccacg tgcgccccat ctccgacgcg gtcctgcgca agtaccgcat cggcaagctc    240
gccgccgccg gcaaggatga gccggccaac gacagcacct actacagctg ggacagcgac    300
ttttacaagg tgctccgcca gcgtgtcgtg gcgcgcctcg aggagcgcaa gatcgcccgc    360
cgcggcggcc ccgagatctg gatcaaggcc gccatcctcg tcagcggctt ctggtccatg    420
ctctacctca tgtgcaccct ggacccgaac cgcggcgcca tcctggccgc catcgcgctg    480
ggcatcgtcg ccgccttcgt cggcacgtgc attcagcacg acggcaacca cggcgcgttc    540
gccttctctc cgttcatgaa caagctctct ggctggacgc tcgacatgat cggcgccagt    600
gccatgacct gggagatgca gcacgtgctg ggccaccacc cgtacaccaa cctgatcgag    660
atggagaacg gcacccaaaa ggtcacccac gccgacgtcg accccaagaa ggccgaccag    720
gagagcgacc cggacgtctt cagcacctac cccatgctcc gtctgcaccc cgtggcaccgc    780
aagcgcttct accaccgctt ccagcacctg tacgcgccgc tgctcttcgg tttcatgacc    840
atcaacaagg tgatcaccca ggatgtggga gttgtcctca gcaagcgtct gtttcagatc    900
gatgccaact gccgttacgc cagcaagtcg tacgttgcgc gcttctggat catgaagctg    960
ctcaccgtcc tctacatggt cgccctcccc gtgtacaccc agggccttgt cgacgggctc    1020
aagctcttct tcatcgccca cttttcgtgc ggcgagctgc tggccaccat gttcatcgtc    1080
aaccacatca tcgagggcgt ctcgtacgcc tccaaggact ctgtcaaggg caccatggcg    1140
ccgccgcgca cggtgcacgg cgtgaccccg atgcatgaca cccgcgacgc gctcggcaag    1200
gagaaggcag ccaccaagca cgtgccgctc aacgactggg ccgcggtcca gtgccagacc    1260
tcggtcaact ggtcgatcgg ctcgtggttc tggaaccact tctccggcgg gctcaaccac    1320
cagatcgagc accacctctt ccccggcctc acccacacca cctacgtgta cattcaggat    1380
gtggtgcagg cgacgtgcgc cgagtacggg gtcccgtacc agtcggagca gagcctcttc    1440
tccgcctact tcaagatgct ctcccacctt cgggcgctcg caacgagcc gatgccctcg      1500
tgggagaagg accacccccaa gtccaagtga                                      1530
```

```
<210> 37
<211> 509
<212> PRT
<213> Schizochytrium aggregatum
```

```
<400> 37
Met Thr Val Gly Gly Asp Glu Val Tyr Ser Met Ala Gln Val Arg Asp
 1               5                   10                  15
His Asn Thr Pro Asp Asp Ala Trp Cys Ala Ile His Gly Glu Val Tyr
             20                  25                  30
Glu Leu Thr Lys Phe Ala Arg Thr His Pro Gly Gly Asp Ile Ile Leu
         35                  40                  45
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Val
     50                  55                  60
Arg Pro Ile Ser Asp Ala Val Leu Arg Lys Tyr Arg Ile Gly Lys Leu
65                  70                  75                  80
Ala Ala Ala Gly Lys Asp Glu Pro Ala Asn Asp Ser Thr Tyr Tyr Ser
                 85                  90                  95
Trp Asp Ser Asp Phe Tyr Lys Val Leu Arg Gln Arg Val Val Ala Arg
            100                 105                 110
Leu Glu Glu Arg Lys Ile Ala Arg Arg Gly Gly Pro Glu Ile Trp Ile
        115                 120                 125
Lys Ala Ala Ile Leu Val Ser Gly Phe Trp Ser Met Leu Tyr Leu Met
    130                 135                 140
Cys Thr Leu Asp Pro Asn Arg Gly Ala Ile Leu Ala Ala Ile Ala Leu
145                 150                 155                 160
Gly Ile Val Ala Ala Phe Val Gly Thr Cys Ile Gln His Asp Gly Asn
                165                 170                 175
His Gly Ala Phe Ala Phe Ser Pro Phe Met Asn Lys Leu Ser Gly Trp
            180                 185                 190
Thr Leu Asp Met Ile Gly Ala Ser Ala Met Thr Trp Glu Met Gln His
```

```
                 195                    200                    205
Val Leu Gly His His Pro Tyr Thr Asn Leu Ile Glu Met Glu Asn Gly
    210                    215                    220
Thr Gln Lys Val Thr His Ala Asp Val Asp Pro Lys Lys Ala Asp Gln
225                    230                    235                    240
Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr Pro Met Leu Arg Leu His
                245                    250                    255
Pro Trp His Arg Lys Arg Phe Tyr His Arg Phe Gln His Leu Tyr Ala
            260                    265                    270
Pro Leu Leu Phe Gly Phe Met Thr Ile Asn Lys Val Ile Thr Gln Asp
        275                    280                    285
Val Gly Val Val Leu Ser Lys Arg Leu Phe Gln Ile Asp Ala Asn Cys
    290                    295                    300
Arg Tyr Ala Ser Lys Ser Tyr Val Ala Arg Phe Trp Ile Met Lys Leu
305                    310                    315                    320
Leu Thr Val Leu Tyr Met Val Ala Leu Pro Val Tyr Thr Gln Gly Leu
                325                    330                    335
Val Asp Gly Leu Lys Leu Phe Phe Ile Ala His Phe Ser Cys Gly Glu
            340                    345                    350
Leu Leu Ala Thr Met Phe Ile Val Asn His Ile Ile Glu Gly Val Ser
        355                    360                    365
Tyr Ala Ser Lys Asp Ser Val Lys Gly Thr Met Ala Pro Pro Arg Thr
    370                    375                    380
Val His Gly Val Thr Pro Met His Asp Thr Arg Asp Ala Leu Gly Lys
385                    390                    395                    400
Glu Lys Ala Ala Thr Lys His Val Pro Leu Asn Asp Trp Ala Ala Val
                405                    410                    415
Gln Cys Gln Thr Ser Val Asn Trp Ser Ile Gly Ser Trp Phe Trp Asn
            420                    425                    430
His Phe Ser Gly Gly Leu Asn His Gln Ile Glu His His Leu Phe Pro
        435                    440                    445
Gly Leu Thr His Thr Thr Tyr Val Tyr Ile Gln Asp Val Val Gln Ala
    450                    455                    460
Thr Cys Ala Glu Tyr Gly Val Pro Tyr Gln Ser Glu Gln Ser Leu Phe
465                    470                    475                    480
Ser Ala Tyr Phe Lys Met Leu Ser His Leu Arg Ala Leu Gly Asn Glu
                485                    490                    495
Pro Met Pro Ser Trp Glu Lys Asp His Pro Lys Ser Lys
            500                    505
```

<210> 38
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO1201

<400> 38
cgtgttcgct gcctttgtcg gaacttgcat cc   32

<210> 39
<211> 33
<212> DNA
<213> Artificial Sequence

<220>

<223> Primer RO1202

<400> 39
ttgacaataa acatggaggc gaggacctct ccg   33

<210> 40
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO1210

<400> 40
gctggttgga ctttggacat gattggatcc   30

<210> 41
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO1211

<400> 41
tacattggca ggccaaccat gtagagaacg   30

<210> 42
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Internal Primer RO1214

<400> 42
ggattcaatc atgtccaaag tccaaccagc   30

<210> 43
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' Primer RO1223

<400> 43
tctgatgaat tcatgacggc cggatttgaa gaag   34

<210> 44
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> 3' Primer RO1224

<400> 44
gtctagctcg agttagttct tgtcccaggc aggca   35

<210> 45
<211> 1542
<212> RNA
<213> Thraustochytrium aureum

<400> 45
atgacggccg gatttgaaga agtgatcacc atgaagcagg tgaaggaccg gaatacgccg   60
gacgatgcgt ggtgcgtggt gcatggcaag gtgtacgaca tcaccaagtt caagaacgct   120
cacccccggtg gagatataat catgttggcg ctggcaaggg acgccaccat cctgttcgag   180
acttaccaca tccgcggtgt gcccgatgcc gtgttgcgca agtatcagat cggcaaactt   240
ccggacggaa agaacaaaga gggcggcaac ggcctcgata gcgcctcgta ctactcctgg   300
gacagcgagt tttaccgcgt ccttcgcgag cgcgtcttga agcgcctgaa cgagctcaag   360
ctgtccagac gcggaggctt cgagatttgg gccaaggcta tctttctctt gaccggcttc   420
tggtcttgcc tctacctcat gtgcacactc aacccaaatg ggcttgcgat tcctgccgcc   480
atgatgttgg gaatctttgc tgccttcgta ggaacctgca ttcagcacga cgggaatcac   540
ggtgcgttcg cccaatcttc gtggcttaac aaggccgctg gttggacttt ggacatgatt   600
ggatccagcg ccatgacctg ggagatgcag cacgtgcttg gacatcatcc gtacaccaac   660
ttgattgaaa tggagaatgg caatcaaaag gtctccggca agcctgttga caccaagact   720
gtcgaccagg agagcgaccc tgatgtcttt agcacctacc ctatgcttcg ccttcaccct   780
tggcacagca aaaagtggta ccacaaatac cagcacatct atgcaccatt catctttggg   840
ttcatgacca tcaacaaggt cattgcacag gacgttggcg ttatcacacg caagcgtctc   900
ttccagattg acgccaactg ccgctacgct tctccgactt acgtcgctcg cttctggatc   960
atgaaggttc ttaccgttct ctacatggtt ggcctgccaa tgtacatgca aggtccatgg   1020
gagggtctca agttgttctt tattgcgcac tttacttgcg gcgagctgct ggccacaatg   1080
ttcatcgtaa accacatcat cgagggtgtc agctacgcaa gcaaagatgc catcaagggc   1140
gagatggctc caccgaaaac ggtccgcggt gtcaccccaa tgcacgagac gcaaaaggtt   1200
ctcgaccagc gcgagaaaga catggacgaa acttctaaga agagccgcat ccctctcaac   1260
gactgggccg ctgtacagtg ccagaccacc gtgaactggg ctatcggttc ttggttctgg   1320
aaccactttt ccgggggcct caatcatcag attgagcatc atctgttccc cggcttgact   1380
cacaccacct atgttcactt tcacgatgtg gtcaaagata cttgcgctga tacggggtt   1440
ccataccagc acgaggagag tctatacact gcctacttta agatgttgaa tcatctcaag   1500
accctaggca acgagccaat gcctgcctgg gacaagaact aa   1542

<210> 46
<211> 513
<212> PRT
<213> Thraustochytrium aureum

<400> 46
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Met Thr Ala Gly Phe Glu Glu Val Ile Thr Met Lys Gln Val Lys Asp
 1               5                  10                  15
Arg Asn Thr Pro Asp Asp Ala Trp Cys Val Val His Gly Lys Val Tyr
            20                  25                  30
Asp Ile Thr Lys Phe Lys Asn Ala His Pro Gly Gly Asp Ile Ile Met
            35                  40                  45
Leu Ala Ala Gly Lys Asp Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
    50                  55                  60
Arg Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Gln Ile Gly Lys Leu
65                  70                  75                  80
Pro Asp Gly Lys Asn Lys Glu Gly Gly Asn Gly Leu Asp Ser Ala Ser
            85                  90                  95
Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg Glu Arg Val
            100                 105                 110
Leu Lys Arg Leu Asn Glu Leu Lys Leu Ser Arg Arg Gly Gly Phe Glu
```

```
          115                  120                  125
Ile Trp Ala Lys Ala Ile Phe Leu Leu Thr Gly Phe Trp Ser Cys Leu
    130                  135                  140
Tyr Leu Met Cys Thr Leu Asn Pro Asn Gly Leu Ala Ile Pro Ala Ala
145                  150                  155                  160
Met Met Leu Gly Ile Phe Ala Ala Phe Val Gly Thr Cys Ile Gln His
              165                  170                  175
Asp Gly Asn His Gly Ala Phe Ala Gln Ser Ser Trp Leu Asn Lys Ala
              180                  185                  190
Ala Gly Trp Thr Leu Asp Met Ile Gly Ser Ser Ala Met Thr Trp Glu
          195                  200                  205
Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu Ile Glu Met
    210                  215                  220
Glu Asn Gly Asn Gln Lys Val Ser Gly Lys Pro Val Asp Thr Lys Thr
225                  230                  235                  240
Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr Pro Met Leu
              245                  250                  255
Arg Leu His Pro Trp His Ser Lys Lys Trp Tyr His Lys Tyr Gln His
          260                  265                  270
Ile Tyr Ala Pro Phe Ile Phe Gly Phe Met Thr Ile Asn Lys Val Ile
        275                  280                  285
Ala Gln Asp Val Gly Val Ile Thr Arg Lys Arg Leu Phe Gln Ile Asp
      290                  295                  300
Ala Asn Cys Arg Tyr Ala Ser Pro Thr Tyr Val Ala Arg Phe Trp Ile
305                  310                  315                  320
Met Lys Val Leu Thr Val Leu Tyr Met Val Gly Leu Pro Met Tyr Met
              325                  330                  335
Gln Gly Pro Trp Glu Gly Leu Lys Leu Phe Phe Ile Ala His Phe Thr
          340                  345                  350
Cys Gly Glu Leu Leu Ala Thr Met Phe Ile Val Asn His Ile Ile Glu
          355                  360                  365
Gly Val Ser Tyr Ala Ser Lys Asp Ala Ile Lys Gly Glu Met Ala Pro
    370                  375                  380
Pro Lys Thr Val Arg Gly Val Thr Pro Met His Glu Thr Gln Lys Val
385                  390                  395                  400
Leu Asp Gln Arg Glu Lys Asp Met Asp Glu Thr Ser Lys Lys Ser Arg
              405                  410                  415
Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr Thr Val Asn
          420                  425                  430
Trp Ala Ile Gly Ser Trp Phe Trp Asn His Phe Ser Gly Gly Leu Asn
        435                  440                  445
His Gln Ile Glu His His Leu Phe Pro Gly Leu Thr His Thr Thr Tyr
    450                  455                  460
Val His Phe His Asp Val Val Lys Asp Thr Cys Ala Glu Tyr Gly Val
465                  470                  475                  480
Pro Tyr Gln His Glu Glu Ser Leu Tyr Thr Ala Tyr Phe Lys Met Leu
              485                  490                  495
Asn His Leu Lys Thr Leu Gly Asn Glu Pro Met Pro Ala Trp Asp Lys
          500                  505                  510
Asn
```

<210> 47
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> M13 Forward Primer

<400> 47
agcggataac aatttcacac agg   23

<210> 48
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO1270

<400> 48
cacctggctc gagtcgacga tgatgg   26

<210> 49
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO1286

<400> 49
cgtacccggt gcaatagaag gtgag   25

<210> 50
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO1287

<400> 50
ccatcatcgt cgactcgagc caggtg   26

<210> 51
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer RO1288

<400> 51
tgtggagcca tgtggtgctc gatctg   26

<210> 52
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Forward Primer RO1400

<400> 52
tcaacagaat tcatgtgcaa cgcggcgcag gtcgagacgc ag   42

<210> 53
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse Primer RO1401

<400> 53
aaaagaaagc ttttagtccg ccttgaccgt gtcgaccaaa gc   42

<210> 54
<211> 1301
<212> DNA
<213> Isochrysis galbana

<400> 54
atgtgcaacg cggcgcaggt cgagacgcag gccttgcgcg ccaaggaggc ggcaaaaccg   60
acgtggacga agattcatgg gcgcacagtc gacgtggaga cgttccgcca cccaggcggc   120
aacatcctcg atttgttcct gggcatggag ccacaactgc ctttgagacg ttccacggtc   180
accacaaggg agcatggaag atgctcaaga cgctgcccga gaaggaggtc gccgccgccg   240
acattcccgc gcagaaggag gagcacgtgg ccgagatgac acgcctcatg gcctcatggc   300
gcgagcgcgg gctgttcaag ccgcgtcccg tcgcctcatc catctatggc ctgtgcgtga   360
tcttcgccat cgcggcatcg gtcgcgtgcg ctccgtacgc gccagtgctg gctggcatcg   420
cggtgggcac ctgctgggct cagtgcggct tcttgcagca catgggcggc caccgggagt   480
gggggcgcac ttggtcgttt gcgtttcagc atctgtttga aggcctgctc aagggcggct   540
cggcctcgtg gtggcgcaac cgccacaaca agcaccatgc caagaccaac gtgctcggcg   600
aggacggcga cctgcgcacc acacccttct cgcatgggga ccctactctg gccaagaaag   660
tgcccgactg gtctctgcgc acgcaagcct tcacctttct gccagcactg ggagcttacg   720
tcttcgtctt tgccttcacg gtacgcaagt acagtgtggt gaagcgtctc tggcacgagg   780
tcgccctgat ggtggcccac tacgctctct tttcctgggc gctcagcgcc gccggcgcct   840
ccctcagctc cggcctcacc ttctattgca ccgggtacgc ctggcagggc atctacctcg   900
gcttcttctt cggcctatcg cactttgcgg tggagcgcgt gccgtcgacc gccacctggc   960
tcgagtcgac gatgatgggc accgttgact ggggcggctc ctccgccttc tgcggctacc   1020
tctccggctt cctcaatatc cagatcgagc accacatggc tccacaaatg ccaatggaga   1080
acctgcgcca gatccgggcc gactgcaagg ccgcggccca caagttcggg ctgccgtacc   1140
gcgagctgac attcgtcgcg gcgaccaagc tcatgatgag cggcctctac cggaccggca   1200
aggacgagct caagctgcgc gcggaccgcc gcaagttcac gagggcacag gcgtacatgg   1260
gcgccgccag cgctttggtc gacacgctca aggcggacta a   1301

<210> 55
<211> 433
<212> PRT
<213> Isochrysis galbana

<400> 55
Met Cys Asn Ala Ala Gln Val Glu Thr Gln Ala Leu Arg Ala Lys Glu
1   5   10   15
Ala Ala Lys Pro Thr Trp Thr Lys Ile His Gly Arg Thr Val Asp Val
            20   25   30
Glu Thr Phe Arg His Pro Gly Gly Asn Ile Leu Asp Leu Phe Leu Gly
        35   40   45
Met Asp Ala Thr Thr Ala Phe Glu Thr Phe His Gly His His Lys Gly

```
        50                      55                      60
Ala Trp Lys Met Leu Lys Thr Leu Pro Glu Lys Glu Val Ala Ala Ala
65                      70                      75                      80
Asp Ile Pro Ala Gln Lys Glu Glu His Val Ala Glu Met Thr Arg Leu
                85                      90                      95
Met Ala Ser Trp Arg Glu Arg Gly Leu Phe Lys Pro Arg Pro Val Ala
            100                     105                     110
Ser Ser Ile Tyr Gly Leu Cys Val Ile Phe Ala Ile Ala Ala Ser Val
            115                     120                     125
Ala Cys Ala Pro Tyr Ala Pro Val Leu Ala Gly Ile Ala Val Gly Thr
    130                     135                     140
Cys Trp Ala Gln Cys Gly Phe Leu Gln His Met Gly Gly His Arg Glu
145                     150                     155                     160
Trp Gly Arg Thr Trp Ser Phe Ala Phe Gln His Leu Phe Glu Gly Leu
                165                     170                     175
Leu Lys Gly Gly Ser Ala Ser Trp Trp Arg Asn Arg His Asn Lys His
            180                     185                     190
His Ala Lys Thr Asn Val Leu Gly Glu Asp Gly Asp Leu Arg Thr Thr
            195                     200                     205
Pro Phe Phe Ala Trp Asp Pro Thr Leu Ala Lys Lys Val Pro Asp Trp
    210                     215                     220
Ser Leu Arg Thr Gln Ala Phe Thr Phe Leu Pro Ala Leu Gly Ala Tyr
225                     230                     235                     240
Val Phe Val Phe Ala Phe Thr Val Arg Lys Tyr Ser Val Val Lys Arg
            245                     250                     255
Leu Trp His Glu Val Ala Leu Met Val Ala His Tyr Ala Leu Phe Ser
            260                     265                     270
Trp Ala Leu Ser Ala Ala Gly Ala Ser Leu Ser Ser Gly Leu Thr Phe
    275                     280                     285
Tyr Cys Thr Gly Tyr Ala Trp Gln Gly Ile Tyr Leu Gly Phe Phe Phe
    290                     295                     300
Gly Leu Ser His Phe Ala Val Glu Arg Val Pro Ser Thr Ala Thr Trp
305                     310                     315                     320
Leu Glu Ser Thr Met Met Gly Thr Val Asp Trp Gly Gly Ser Ser Ala
            325                     330                     335
Phe Cys Gly Tyr Leu Ser Gly Phe Leu Asn Ile Gln Ile Glu His His
            340                     345                     350
Met Ala Pro Gln Met Pro Met Glu Asn Leu Arg Gln Ile Arg Ala Asp
            355                     360                     365
Cys Lys Ala Ala Ala His Lys Phe Gly Leu Pro Tyr Arg Glu Leu Thr
    370                     375                     380
Phe Val Ala Ala Thr Lys Leu Met Met Ser Gly Leu Tyr Arg Thr Gly
385                     390                     395                     400
Lys Asp Glu Leu Lys Leu Arg Ala Asp Arg Arg Lys Phe Thr Arg Ala
            405                     410                     415
Gln Ala Tyr Met Gly Ala Ala Ser Ala Leu Val Asp Thr Leu Lys Ala
            420                     425                     430
Asp
```

```
<210> 56
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Protein Motif #1
```

```
<400> 56
Val Tyr Asp Val Thr Glu Trp Val Lys Arg His Pro Gly Gly
 1               5                   10


<210> 57
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Protein Motif #2

<400> 57
Gly Ala Ser Ala Asn Trp Trp Lys His Gln His Asn Val His His
 1               5                   10                  15


<210> 58
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Protein Motif #3

<400> 58
Asn Tyr Gln Ile Glu His His Leu Phe Pro Thr Met
 1               5                   10


<210> 59
<211> 6
<212> PRT
<213> Thraustochytrium aureum

<400> 59
Gln His Asp Gly Ser His
 1               5


<210> 60
<211> 7
<212> PRT
<213> Thraustochytrium aureum

<400> 60
Gln His Val Leu Gly His His
 1               5


<210> 61
<211> 4
<212> PRT
<213> Thraustochytrium aureum

<400> 61
His Pro Trp His
 1


<210> 62
```

```
<211> 5
<212> PRT
<213> Thraustochytrium aureum

<400> 62
His Lys Phe Gln His
 1               5


<210> 63
<211> 6
<212> PRT
<213> Thraustochytrium aureum

<400> 63
His Gln Ile Glu His His
 1               5


<210> 64
<211> 7
<212> PRT
<213> Dictyostelium discoideum

<400> 64
Gln His Val Ile Gly His His
 1               5


<210> 65
<211> 6
<212> PRT
<213> Dictyostelium discoideum

<400> 65
His Gln Val Val His His
 1               5


<210> 66
<211> 7
<212> PRT
<213> Mortierella alpina

<400> 66
Gln His Met Leu Gly His His
 1               5


<210> 67
<211> 6
<212> PRT
<213> Unknown

<220>
<223> Synechocytis (species unknown)

<400> 67
His Gln Val Thr His His
 1               5


<210> 68
```

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<211> 335
<212> PRT
<213> Schizochytrium aggregatum

<220>
<221> VARIANT
<222> (335)...(335)
<223> Xaa = Unknown or other at position 335

<400> 68
Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met Asn Lys Ala Ala Gly
1               5                   10                  15
Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met Thr Trp Glu Met Gln
            20                  25                  30
His Val Leu Gly His His Pro Tyr Thr Asn Leu Ile Glu Met Glu Asn
        35                  40                  45
Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp Pro Lys Lys Val Asp
    50                  55                  60
Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr Pro Met Leu Arg Leu
65              70                  75                      80
His Pro Trp His Arg Gln Arg Phe Tyr His Lys Phe Gln His Leu Tyr
            85                  90                  95
Ala Pro Leu Ile Phe Gly Phe Met Thr Ile Asn Lys Val Ile Ser Gln
        100                 105                 110
Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe Gln Ile Asp Ala Asn
        115                 120                 125
Cys Arg Tyr Gly Ser Pro Trp Asn Val Ala Arg Phe Trp Ile Met Lys
    130                 135                 140
Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro Met Tyr Met Gln Gly
145                 150                 155                 160
Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala His Phe Thr Cys Gly
            165                 170                 175
Glu Val Leu Ala Thr Met Phe Ile Val Asn His Ile Ile Glu Gly Val
        180                 185                 190
Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val Met Ala Pro Pro Arg
        195                 200                 205
Thr Val His Gly Val Thr Pro Met Gln Val Thr Gln Lys Ala Leu Ser
    210                 215                 220
Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys Thr Thr Met Ile Pro
225                 230                 235                 240
Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr Ser Val Asn Trp Ala
            245                 250                 255
Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly Gly Leu Asn His Gln
            260                 265                 270
Ile Glu His His Cys Phe Pro Gln Asn Pro His Thr Val Asn Val Tyr
    275                 280                 285
Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu Tyr Gly Val Pro Tyr
    290                 295                 300
Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe Lys Met Leu Ser His
305                 310                 315                 320
Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala Trp Ser Thr Xaa
            325                 330                 335

<210> 69
<211> 430
<212> PRT
<213> Isochrysis galbana
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```
<400> 69
Met Cys Asn Ala Ala Gln Val Glu Thr Gln Ala Leu Arg Ala Lys Glu
1               5                   10                  15
Ala Ala Lys Pro Thr Trp Thr Lys Ile His Gly Arg Thr Val Asp Val
            20                  25                  30
Glu Thr Phe Arg His Pro Gly Gly Asn Ile Leu Asp Leu Phe Leu Gly
        35                  40                  45
Met Asp Ala Thr Thr Ala Phe Glu Thr Phe His Gly His His Lys Gly
    50                  55                  60
Ala Trp Lys Met Leu Lys Thr Leu Pro Glu Lys Glu Val Ala Ala Ala
65                  70                  75                  80
Asp Ile Pro Ala Gln Lys Glu Glu His Val Ala Glu Met Thr Arg Leu
                85                  90                  95
Met Ala Ser Trp Arg Glu Arg Gly Leu Phe Lys Pro Arg Pro Val Ala
            100                 105                 110
Ser Ser Ile Tyr Gly Leu Cys Val Ile Phe Ala Ile Ala Ala Ser Val
        115                 120                 125
Ala Cys Ala Pro Tyr Ala Pro Val Leu Ala Gly Ile Ala Val Gly Thr
    130                 135                 140
Cys Trp Ala Gln Cys Gly Phe Leu Gln His Met Gly Gly His Arg Glu
145                 150                 155                 160
Trp Gly Arg Thr Trp Ser Phe Ala Phe Gln His Leu Phe Glu Gly Leu
                165                 170                 175
Leu Lys Gly Gly Ser Ala Ser Trp Trp Arg Asn Arg His Asn Lys His
            180                 185                 190
His Ala Lys Thr Asn Val Leu Gly Glu Asp Gly Asp Leu Arg Thr Thr
            195                 200                 205
Pro Phe Phe Ala Trp Asp Pro Thr Leu Ala Lys Lys Val Pro Asp Trp
    210                 215                 220
Ser Leu Arg Thr Gln Ala Phe Thr Phe Leu Pro Ala Leu Gly Ala Tyr
225                 230                 235                 240
Val Phe Val Phe Ala Phe Thr Val Arg Lys Tyr Ser Val Val Lys Arg
            245                 250                 255
Leu Trp His Glu Val Ala Leu Met Val Ala His Tyr Ala Leu Phe Ser
            260                 265                 270
Trp Ala Leu Ser Ala Ala Gly Ala Ser Leu Ser Ser Gly Leu Thr Phe
    275                 280                 285
Tyr Cys Thr Gly Tyr Ala Trp Gln Gly Ile Tyr Leu Gly Phe Phe Phe
    290                 295                 300         -
Gly Leu Ser His Phe Ala Val Glu Arg Val Pro Ser Thr Ala Thr Trp
305                 310                 315                 320
Leu Glu Ser Thr Met Met Gly Thr Val Asp Trp Gly Gly Ser Ser Ala
            325                 330                 335
Phe Cys Gly Tyr Leu Ser Gly Phe Leu Asn Ile Gln Ile Glu His His
            340                 345                 350
Met Ala Pro Gln Met Pro Met Glu Asn Leu Arg Gln Ile Arg Ala Asp
    355                 360                 365
Cys Lys Ala Ala Ala His Lys Phe Gly Leu Pro Tyr Arg Glu Leu Thr
    370                 375                 380
Phe Val Ala Ala Thr Lys Leu Met Met Ser Gly Leu Tyr Arg Thr Gly
385                 390                 395                 400
Lys Asp Glu Leu Lys Leu Arg Ala Asp Arg Arg Lys Phe Thr Arg Ala
            405                 410                 415
Gln Ala Tyr Met Gly Ala Ala Ser Ala Leu Val Asp Thr Leu
    420                 425                 430
```

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<210> 70
<211> 515
<212> PRT
<213> Thraustochytrium aureum

<400> 70
Met Thr Val Gly Phe Asp Glu Thr Val Thr Met Asp Thr Val Arg Asn
 1               5                   10                  15
His Asn Met Pro Asp Asp Ala Trp Cys Ala Ile His Gly Thr Val Tyr
            20                  25                  30
Asp Ile Thr Lys Phe Ser Lys Val His Pro Gly Gly Asp Ile Ile Met
            35                  40                  45
Leu Ala Ala Gly Lys Glu Ala Thr Ile Leu Phe Glu Thr Tyr His Ile
        50                  55                  60
Lys Gly Val Pro Asp Ala Val Leu Arg Lys Tyr Lys Val Gly Lys Leu
65                  70                  75                  80
Pro Gln Gly Lys Lys Gly Glu Thr Ser His Met Pro Thr Gly Leu Asp
                85                  90                  95
Ser Ala Ser Tyr Tyr Ser Trp Asp Ser Glu Phe Tyr Arg Val Leu Arg
            100                 105                 110
Glu Arg Val Ala Lys Lys Leu Ala Glu Pro Gly Leu Met Gln Arg Ala
        115                 120                 125
Arg Met Glu Leu Trp Ala Lys Ala Ile Phe Leu Leu Ala Gly Phe Trp
    130                 135                 140
Gly Ser Leu Tyr Ala Met Cys Val Leu Asp Pro His Gly Gly Ala Met
145                 150                 155                 160
Val Ala Ala Val Thr Leu Gly Val Phe Ala Ala Phe Val Gly Thr Cys
                165                 170                 175
Ile Gln His Asp Gly Ser His Gly Ala Phe Ser Lys Ser Arg Phe Met
            180                 185                 190
Asn Lys Ala Ala Gly Trp Thr Leu Asp Met Ile Gly Ala Ser Ala Met
            195                 200                 205
Thr Trp Glu Met Gln His Val Leu Gly His His Pro Tyr Thr Asn Leu
    210                 215                 220
Ile Glu Met Glu Asn Gly Leu Ala Lys Val Lys Gly Ala Asp Val Asp
225                 230                 235                 240
Pro Lys Lys Val Asp Gln Glu Ser Asp Pro Asp Val Phe Ser Thr Tyr
                245                 250                 255
Pro Met Leu Arg Leu His Pro Trp His Arg Gln Arg Phe Tyr His Lys
            260                 265                 270
Phe Gln His Leu Tyr Ala Pro Leu Ile Phe Gly Phe Met Thr Ile Asn
            275                 280                 285
Lys Val Ile Ser Gln Asp Val Gly Val Val Leu Arg Lys Arg Leu Phe
        290                 295                 300
Gln Ile Asp Ala Asn Cys Arg Tyr Gly Ser Pro Trp Asn Val Ala Arg
305                 310                 315                 320
Phe Trp Ile Met Lys Leu Leu Thr Thr Leu Tyr Met Val Ala Leu Pro
                325                 330                 335
Met Tyr Met Gln Gly Pro Ala Gln Gly Leu Lys Leu Phe Phe Met Ala
            340                 345                 350
His Phe Thr Cys Gly Glu Val Leu Ala Thr Met Phe Ile Val Asn His
            355                 360                 365
Ile Ile Glu Gly Val Ser Tyr Ala Ser Lys Asp Ala Val Lys Gly Val
    370                 375                 380
Met Ala Pro Pro Arg Thr Val His Gly Val Thr Pro Met Gln Val Thr
385                 390                 395                 400
Gln Lys Ala Leu Ser Ala Ala Glu Ser Thr Lys Ser Asp Ala Asp Lys
```

```
                   405                   410                   415
Thr Thr Met Ile Pro Leu Asn Asp Trp Ala Ala Val Gln Cys Gln Thr
            420                   425                   430
Ser Val Asn Trp Ala Val Gly Ser Trp Phe Trp Asn His Phe Ser Gly
            435                   440                   445
Gly Leu Asn His Gln Ile Glu His His Cys Phe Pro Gln Asn Pro His
        450                   455                   460
Thr Val Asn Val Tyr Ile Ser Gly Ile Val Lys Glu Thr Cys Glu Glu
465                   470                   475                   480
Tyr Gly Val Pro Tyr Gln Ala Glu Ile Ser Leu Phe Ser Ala Tyr Phe
                485                   490                   495
Lys Met Leu Ser His Leu Arg Thr Leu Gly Asn Glu Asp Leu Thr Ala
            500                   505                   510
Trp Ser Thr
        515
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```
<210> 71
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Histidine-Rich Motif

<400> 71
His Met Gly Gly His
 1               5

<210> 72
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Histidine-Rich Motif

<400> 72
His Asn Lys His His
 1               5

<210> 73
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Histidine-Rich Motif

<400> 73
Gln Ile Glu His His
 1               5
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