



US 20030152983A1

(19) **United States**(12) **Patent Application Publication****Napier et al.**(10) **Pub. No.: US 2003/0152983 A1**(43) **Pub. Date: Aug. 14, 2003**(54) **DESATURASE**(30) **Foreign Application Priority Data**(75) Inventors: **Johnathan A. Napier**, Bristol (GB);
Louise Michaelson, Bristol (GB);
Keith Stobart, Bristol (GB)Dec. 23, 1997 (GB) 9727256.1
Jun. 29, 1998 (GB) 9814034.6

Correspondence Address:

BANNER & WITCOFF**1001 G STREET N W****SUITE 1100****WASHINGTON, DC 20001 (US)****Publication Classification**(51) **Int. Cl.⁷** **C12Q 1/68**; C07H 21/04;
C12N 9/04; C12N 1/18; C12N 15/82;
A01H 1/00(52) **U.S. Cl.** **435/6**; 435/69.1; 435/190;
435/320.1; 435/325; 536/23.2;
435/254.2; 800/281(73) Assignee: **University of Bristol**, Bristol (GB)(21) Appl. No.: **10/340,779**(22) Filed: **Jan. 13, 2003**(57) **ABSTRACT****Related U.S. Application Data**(63) Continuation of application No. 09/582,034, filed on
Dec. 19, 2000, filed as 371 of international applica-
tion No. PCT/GB98/03895, filed on Dec. 23, 1998.This invention relates to cDNA sequences encoding Δ^5 -fatty
acid desaturases comprising the sequences shown in SEQ.1
and SEQ.2.

S54259	:	-----	:	-
S54809	:	-----	:	-
S68358	:	MVSPSIEVLNSIADGKKYITSKELKKHNNPNDLWIS	:	36
S35157	:	-----	:	-
PBOR6	:	-----MAAQIKKYITSDELKNHDKPGDLWIS	:	26
FU2	:	-----MGTDQGKTFTWEELAAHNTKGDFLA	:	26

				T
S54259	:	-----MTLSIVK	:	7
S54809	:	-----MTSTTSKVT	:	9
S68358	:	ILGKVYNVTEWAKEHPGGDAPLINLAGQDVDAFLA	:	72
S35157	:	-----MLTAERIK	:	8
PBOR6	:	IQGKAYDVSDWVKDHPGGSFPLKSLAGQEVDAFVA	:	62
FU2	:	IRGRVYDVTKFLSRHPGGVDLLLGAAGRDVTPVFEM	:	62

S54259	:	SEDS---SSRPSAVPSDLPLEEDIINTLPSG----	:	35
S54809	:	EGKS-IGFRKELNRRVNAYLEAENISP--RD-----	:	37
S68358	:	FHPG-TAKKHLDKLFTGYHLEKDYQVSDISRDRKLA	:	107
S35157	:	ETQK-RGFRRVLNQRVDAYFAEHGLTQ--RD-----	:	36
PBOR6	:	FHPA-STWKNLDKFFTGYYLKDYSEVSKBYRKL	:	97
FU2	:	YHAFGAADAIMKKYYVGTLVSNELPVFPEPTVFHKT	:	98

		5		6
S54259	:	--VFVQDRYKA-----WMTVLIINVIMVG	:	56
S54809	:	---NPPMYLK-----TAIILAWVVS	:	54
S68358	:	SEFAKAGMFEKKGH-----GVIYELCFVSLILS	:	135
S35157	:	---NPSMYLK-----TLIIVLWLFSS	:	53
PBOR6	:	FEFSKMGLYDKKGH-----IMFATLCFLAMEFA	:	125
FU2	:	IKTRVEGYETDRDIDPKNRPEIWGRYALIFGSLIAS	:	134

		6	6	g
S54259	:	--LGWLGIAIAPWFLLPVVW-VFTGTALTGFFV-IG	:	88
S54809	:	--AWTEVVFGPDVLWMKLLGCIVLGFGVSAVGFNIS	:	88
S68358	:	--ACVYGVLYSGSFWIHMLSGAILGLAWMQIAY-IG	:	168
S35157	:	--AWAEVLFAPVIFPVRLGCMVLAIALAASFENVG	:	87
PBOR6	:	--MSVYGVLFCEGVLVHLFSGCLMGFLWIQSGW-IG	:	158
FU2	:	YYAQLEVPFVVERTWLQVVFATIMGFACAQVGLNPL	:	170

FIG. 1

	HD	H	n	G	g	s	W	
S54259	:	HDCG	HRSF	SRNV	VNDW	VGHILF	-LP	I I Y P F H S W R I : 123
S54809	:	HDGN	HGGYSKYQ	WNYL	SG	LTHD	--A	IGVESYLWKF : 122
S68358	:	HDAG	HYQMMAT	RGW	NKFAG	EFIGNC	ITG	ISIAWKKW : 204
S35157	:	HDAN	ENAYSS	NPHIN	RVL	GMTYD	--F	VGLSSFLWRY : 121
PBOR6	:	HDAG	HYMVVSD	SR	LNKFM	GIFA	ANCL	SGISIGWKKW : 194
FU2	:	HDAS	HFSV	THNPT	VWKIL	GATHD	-FF	NGASYLVNMY : 205

	Hn	HH	N	D					
S54259	:	GHN	-QHHKY	TNR	MELN	AWQ---PWRKE----	E--- : 148		
S54809	:	RHN	VLHHTY	TN	ILGH	DEVE	TH-GDELVRMS	PSME--Y : 155	
S68358	:	TNN	-AHH	IACNS	LDYD	PD	LQ-HLEMLAV	SSKLFNSI : 238	
S35157	:	RHN	YLHHTY	TN	ILGH	DEVE	TH-GDGAVRMS	PEQE--H : 154	
PBOR6	:	NNN	-AHH	IACNS	LEYD	PD	LQ-YIEFLV	VS	SKFFGSL : 228
FU2	:	QHML	GHH	PHY	TN	IAGAD	PDVSTFE	EDVRR	IKPNQ--K : 239

	5	6													
S54259	:	----	YONAGKEMQ	VTYDL	FR	GRA	WIGS	ILH	WAS	TH : 180					
S54809	:	R-WY	HRYQ	HWE	TFV	Y	PE	I	PY-Y	NSIAD	VQ	TML	FKR : 189		
S68358	:	TSV	FYGR	LT	ED	PLAR	FE	VSY	QH	LY	PIM	CA	ARIN : 274		
S35157	:	V-GI	YRFQ	QFY	WGL	YLE	I	PE-Y	WFLY	DV	YL	VL	NKG : 188		
PBOR6	:	TSH	FYE	KRL	T	ED	SL	SR	FE	VSY	QH	WTF	PIM	CA	ARIN : 264
FU2	:	WFV	NH	IN	QD	ME	V	PFL	XGL	LAF-KV	R	IQD	IN	ILY	FVK : 274

	P																														
S54259	:	-EDWTKFE	GKQRQ	QVK-F	SSLL	VIG	AA	IAF--	PTM : 212																						
S54809	:	QYHD	HE	IP	SP	TW	VD	IAT	LLA	FA	FG	VAV	FLI-IP	IA : 224																	
S68358	:	L	YL	Q	T	I	L	L	L	I	S	K	R	KIP-DR	GLN	IL	G	T	L	I	F	W	T	W	F	ELL : 309					
S35157	:	KYHD	H	K	T	P	F	Q	P	L	E	I	A	S	L	L	G	I	K	L	W	L	G	Y	V	F	G-L	PLA : 223			
PBOR6	:	MYV	Q	S	L	I	M	L	L	T	K	R	N	Y	S-Y	R	A	H	E	L	L	G	C	L	V	F	S	I	W	Y	ELL : 299
FU2	:	TN	D	A	I	R	V	N	P	I	S	T	W	H	T	V	M	F	W	G	G	K	A	F	F	W	Y	R	L	I-V	PLQ : 309

	6	f	l	H																															
S54259	:	ILT	I	G-V	W	G	F	V	K	F	W	V	I	P	W	L	V	F	H	F	W	M	S	T	F	T	L	L	H	R	T	I	A : 247		
S54809	:	V	G	Y	S	P--	L	E	A	V	I	G	A	S	I	V	M	T	H	G	L	V	A	C	V	V	E	M	L	A	H	V	I	E : 258	
S68358	:	V	S	R	L	P	N	W	P	E	R	V	A	F	V	L	V	S	E	C	V	T	G-I	Q	H	I	Q	E	T	L	N	H	F	S	G : 344
S35157	:	L	G	F	S	I--	P	E	V	L	I	G	A	S	V	T	Y	M	T	Y	G	I	V	V	C	T	I	E	M	L	A	H	V	I	E : 257
PBOR6	:	V	S	C	L	P	N	W	G	E	R	I	M	F	V	I	A	S	L	S	V	T	G-M	Q	Q	V	Q	F	S	I	N	H	F	S	S : 334
FU2	:	Y	L	P	L	G---	K	V	L	L	L	F	T	Y	A	D	M	V	S	S	Y	W	L	A	L	T	E	Q	A	N	H	V	E : 342		

FIG. 1 CONT'D

	P	W	2	3	
S54259	:	DIPFERPE-----	QWHEAESQLSGTVHCNYSRW	:	275
S54809	:	PAEFLDPD--NLHIDDEWAIAQVKTTVDFAPN-NPI	:	291	
S68358	:	DVYVGPEKG-----	DNWFEKQTRGTIDIA-C-SSW	:	372
S35157	:	STEELTPDGESGAIDDEWAICQIRTTANFATN-NPF	:	292	
PBOR6	:	SVYVGKEKG-----	NNWFEKQTDGTLDIS-C-PPW	:	362
FU2	:	EVQWPLPDE-NGIIQKDWAAMQVETTDYAHQ-SHL	:	376	

	g	6	q	HH6fp	6	C
S54259	:	GEFLCHDINVH	IPHRVTTA	IPWYNLRTPT	TPVYRKIG	: 311
S54809	:	INWYVGGLNYQ	TVHHLEPH	ICH	IHYPKIAPILA	EV : 327
S68358	:	MDWFFGGLOFQ	LEHHELEP	R	LRCHLSISPICRE	LC : 408
S35157	:	WNWECGGLNHQ	VTHHLEP	N	ICH	IHYPOLENI : 328
PBOR6	:	MDWFHGGLOFQ	LEHHELEP	K	MPCNLRKIS	EVVIELC : 398
FU2	:	WTSITGSLNYQ	AVHHELEP	N	VSQHHPDILAI	IKNTC : 412

	Y	f	a	6
S54259	:	GEYLYPECDF	SWGLMKQV	VDHATCMRITITSQSLT : 347
S54809	:	EEFGVNYA--	VHOTTEFG	AALANYSWLKKMSINP--E : 359
S68358	:	KKYNLPY---	VSLSEYDAN	VTTIKTLRTAAIQARDL : 441
S35157	:	QEEGVEYK--	VYPTEKAA	IASNYRWLEAMGKAS--- : 359
PBOR6	:	KKHNLPY---	NYASTSKAN	EMTERTLRNTALQARDI : 431
FU2	:	SEYKVEYL--	VKDTEWQAF	ASHLEHLRVLGIRPKEE : 446

S54259	:	TKRV-----	:	351
S54809	:	TKAIEQLTV-----	:	368
S68358	:	TNPAPQNLAW	EAFNTH-G	: 458
S35157	:	-----	:	-
PBOR6	:	TKPLPKNLV	WEALHTHG-	: 448
FU2	:	-----	:	-

S54259	Δ^{12} <i>Spirulina</i>
S54809	Δ^6 <i>Spirulina</i>
S68358	Putative Sphingolipid desaturase
S35157	Δ^6 <i>Synechocystis</i>
PBOR6	Δ^6 Borage
FU2	Δ^5 desaturase

FIG. 1 CONT'D

FIG. 2

An alignment of the deduced amino acid sequences of the Δ^5 fatty acid desaturase from *C.elegans* with the *C.elegans* Δ^6 fatty acid desaturase and the Δ^5 fatty acid desaturase from the fungus *M.alpina*.

```

CE5   1  -----MVLREQEHEPFFTKIDGRWCQIDDAVLRSHPGGS-AITTYKMDATTV
CE6   1  -----MVVDKN-ASGLRMKVVDGKWLVLSEELVKKHPPGA-VIEQYRNSDATHI
MA5   1  MGTDQGKTFTWEEELAAHNTKGDLELAIRGRVYDVTKFLSR-HPGGVDTILLGAGRIVTPV

CE5  48  FHTTHT-GSKRAYQWLETELKECPTQEPPIPDIKDEPIKGIDDVNMGTINISEKRSAGIN
CE6  47  FHAFFE-GSSQAYKOLDLLKHHG--EHDZFLKQLEKRLDKVDINVSAYDVSVAQEKKMV
MA5  60  FEMYHAFGAADAIMKKYYVGTLSNLELVFPEP-----T-----V--FHKTKTRVEGY

CE5 107  KSETDLRMKVRAEGHMDGSPLEFYTRKILETIFTTLFAFYLQYHTTYLPSATIMAVANDQIL
CE6 104  ESEKLNQKLEHDDGLMKANETTFLEFKATSTESIMAFAYLOELGWETTSACILALARGQF
MA5 107  FEDRDIDFKNRPE-IWGRYALIEGSLIASYYAQLFVPEVVERTWLQVVFATIMIFACAGV

CE5 167  GWLI-HEFAHHQLFNRYYNDLASYEVGNFLQGFBSGGWKEQINV-HBAATNVVGRDCHL
CE6 164  GWLT-HEFCHQOPTKNRPLNDTISLEFFGNFLQGFSDRWWRDKHNT-HBAATNVVDHDCOI
MA5 166  GLNPLHDASHFSVTHNP-TVWKILGATHDEFFNGASYLVVMYQHMIGHHPYTNLAG--AE--

CE5 225  DLVEFYATVAEHLNNSQ--DSWVMTLFRNORVHWTTFMLEFFLRLSWLLQSTIEFSOMPTH
CE6 222  DLAPLFATIPGDLCKYRASFEKAILKIVPYQHLYFTAMLEPMKRTSWTGQSVQVVEKENOM
MA5 222  ---PDVSTFEFDVRRIKPN-QKWFVNHN-DMNEVPTLYGLAEKVRIDINILEYTKIN

CE5 283  YDYXRNTALYEDVGLSLHWANSLGOLYFLEDNST----KIMTFLVSHLVGGFLSRYVCT
CE6 282  EYKVYQRNAPWEQATVGHWANVFTQLFLEETPL----KVATFELSOMGGGLTARVVT
MA5 277  DAIRVNPISTWHTVMFWGGKATFVWYRLIVPQYLPGLGVILLETVADMVSSYWLALTFQ

CE5 339  ENHYSEVERFALSN----TMSNYACLOIMTTRNMRP-GRFIDNLWGGGLNYQIEHRLFTM
CE6 338  ENHNSVDKYPANR----ILNNFAALOILTTRNMTB-SPEIDNLWGGGLNYQIEHRLFTM
MA5 337  ANHVVEEVQWPLPDENGIIQKDNAAMQVETFOYAHDSHLWTSITGSINYGAVHRLFPNV

CE5 394  PRHNENTVMPLVKEFAAANGLPYNDV--YFTGEWLEIEQFRNIANVAAKLTKKIA
CE6 393  PECHLNACVKVKEWCKENNLPYLVDV--YEDGYAMNLOQLKMAEHIQAKA---
MA5 397  SQHHYPDILLATIKNTCSYKVPYLVKETFWQAFASHLEHLRVLGLRPKEE-----

```

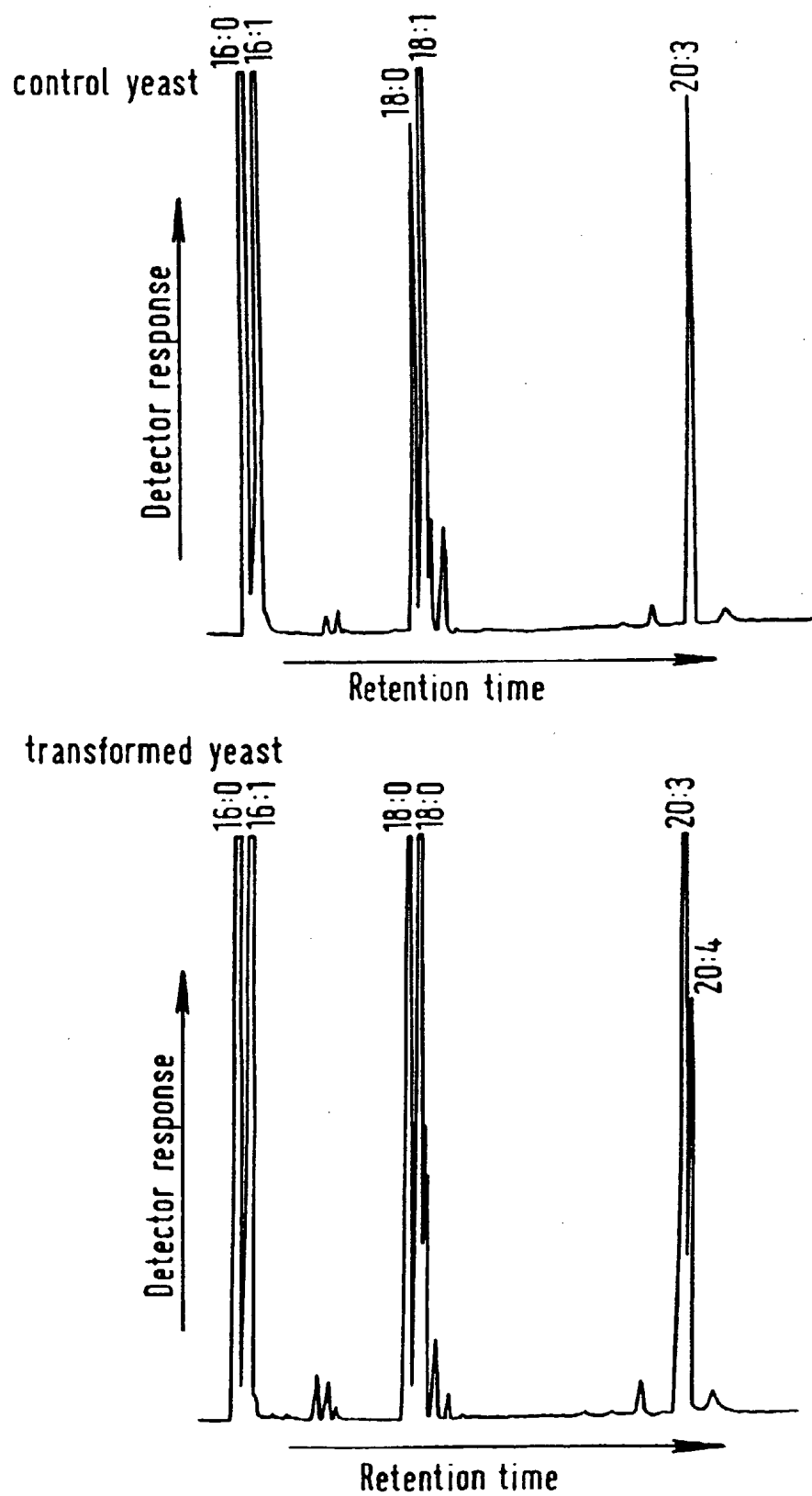


FIG. 3

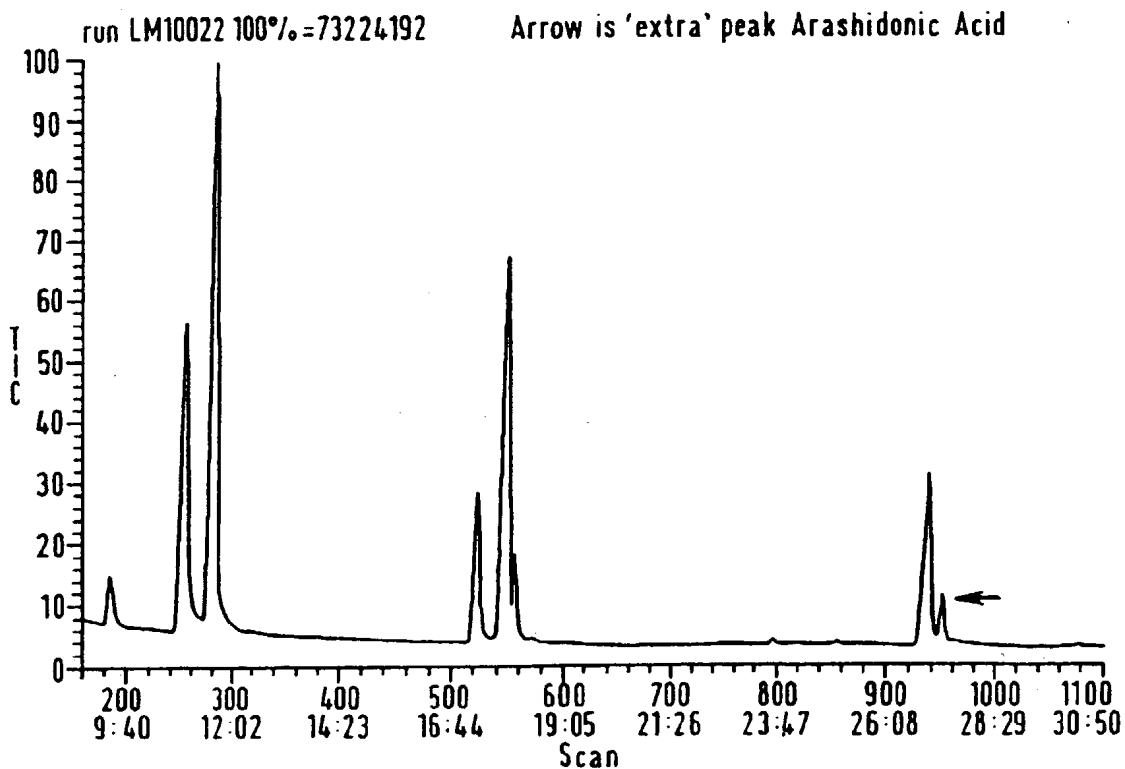
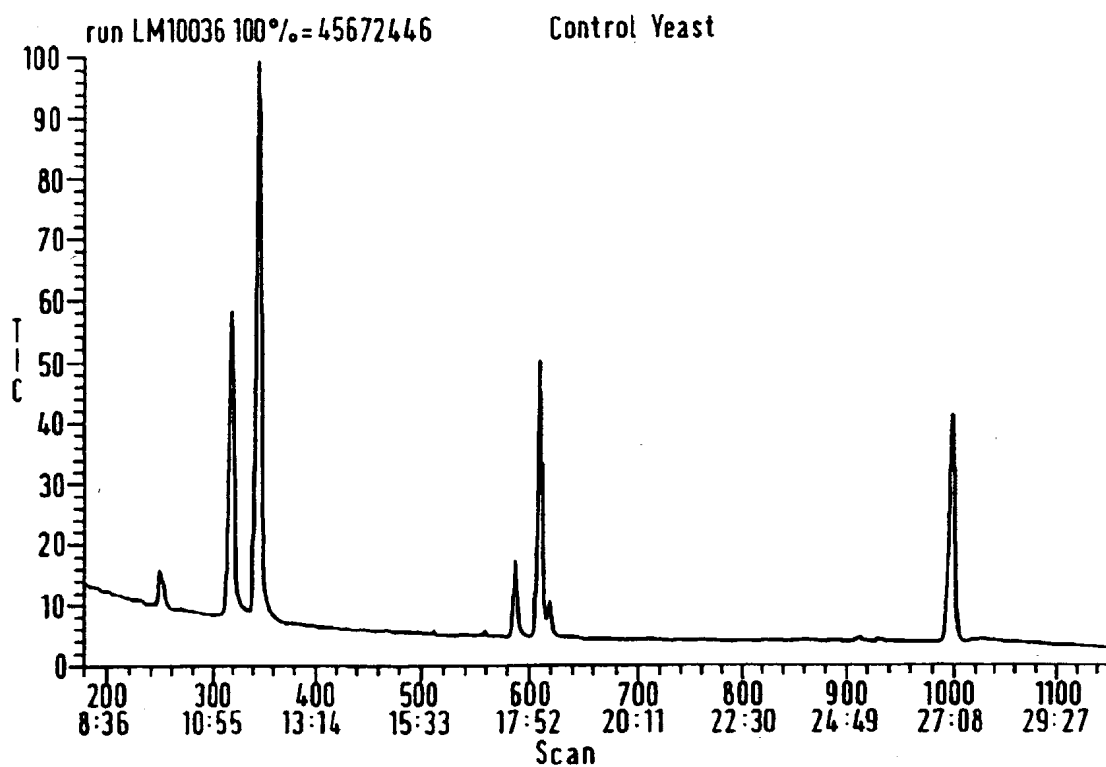


FIG. 4.

DESATURASE

[0001] This invention relates to DNA sequences encoding $\Delta 5$ -fatty acid desaturases, the encoded $\Delta 5$ -fatty acid desaturases, and applications for the $\Delta 5$ -fatty acid desaturases.

[0002] Polyunsaturated fatty acids are important neutraceutically due to their specific health promoting activities, and biomedically in respect of their potential pharmaceutical applications in the treatment of specific disease conditions.

[0003] Polyunsaturated fatty acids are the precursors for two major classes of metabolites: prostanoids (which include prostaglandins and thromboxanes) and leukotrienes. $\Delta 5$ -fatty acid desaturase catalyses the conversion of dihomogammala linolenic acid (DHL) to arachidonic (AA) acid, and eicosatetraenoate (ETA) to ecosapentaenoate (EPA), by the introduction of double bonds at the $\Delta 5$ carbon of the respective substrates, and exists as an endoplasmic reticulum membrane-bound protein in its native state.

[0004] Arachidonic acid has a 20 carbon chain with 4 double bonds and is of great importance in human metabolism since it is a precursor for the synthesis of prostaglandins—20-carbon chain fatty acids that contain a 5 carbon ring. Prostaglandins are modulators of hormone action and the potential effects of prostaglandins include the stimulation of inflammation, the regulation of blood flow to particular organs, the control of ion transport across some membranes, and the modulation of synaptic transmission. Prostaglandins are also potentially useful as contraceptives due to their ability to suppress progesterone secretion. Therefore, the ability to modulate prostaglandin synthesis by controlled levels of expression of polyunsaturated fatty acid precursor synthesis is very important both medically and commercially.

[0005] The increased importance of polyunsaturated fatty acids in the food and pharmaceutical industries has led to an increased demand which has exceeded current production levels and supplementary sources of high quality, low cost polyunsaturated fatty acids are required.

[0006] Current commercial sources of polyunsaturated fatty acids include selected seed plants, marine fish and selected mammals, and traditional processing techniques for extracting the polyunsaturated fatty acids from these sources include solvent extraction, winterization, urea-adduct formation and distillation. However, present sources have the disadvantages of seasonal and climatic variations in both production levels and quality, a lack of availability of plant and fish sources, and the high costs of refining low-grade oils. High costs coupled with insufficient production levels have retarded the development of commercially exploitable applications of polyunsaturated fatty acids.

[0007] Much effort has gone into developing alternative sources of polyunsaturated fatty acids, and studies have been carried out to characterise the constituent genes and encoded proteins of their biosynthesis. The engineering of polyunsaturated fatty acid biosynthesis into oilseeds for example has many advantages for the production of large scale quantities of, for example, γ -linolenate (GLA), dihomogammala linolenate (DHGLA), arachidonic acid (AA), ecosapentaenoate (EPA) and docosaheptaenoate (DHA). The practicality of this has been illustrated by the expression of a Borage $\Delta 6$ desaturase gene in tobacco resulting in the production of GLA and the octadecatetraenoic acid, 18:4

(Soyanova et al (1997), *PNAS* 94, 9411-9414). As more of the biosynthetic genes for polyunsaturated fatty acid synthesis become available, this opens up the possibility of producing at least GLA, AA, EPA and DHA in oil seeds, as well as controlling the type of lipid assembled. Benefits which would be obtained from such crops include a cheap and sustainable supply of desirable polyunsaturated fatty acids on a large scale, tailored polyunsaturated fatty acids profiles to meet specific nutritional requirements, and in the fine chemical industry, the production of unusual fatty acids with prescribed levels and locations of unsaturation.

[0008] A further approach to the production of polyunsaturated fatty acids is to utilise the biosynthetic capacity of lower organisms e.g. algae, bacteria, fungi (including phycomycetes) which can synthesise the entire range of polyunsaturated fatty acids and can be grown on an industrial scale. Genetic transformation of these organisms will enable the development of overproducing strains and the manipulation of the polyunsaturated profile by pathway engineering.

[0009] Fungal $\Delta 5$ and $\Delta 6$ fatty acid desaturases have been cloned, and their sequences disclosed in WO98/46763, WO98/46764 and WO98/46765.

[0010] Polyunsaturated fatty acid metabolism is of greatest importance in human metabolism. These acids, via the eicosanoids, are fundamental to the proper maintenance of homeostasis and are linked to serious physiological and pathophysiological syndromes.

[0011] The inventors have surprisingly isolated and characterised a DNA sequence from the soil-borne filamentous fungus of the zygomycete class *Mortierella alpina* encoding a functional $\Delta 5$ -fatty acid desaturase.

[0012] In addition, the inventors have surprisingly isolated and characterised a DNA sequence from the nematode worm, *Caenorhabditis elegans* encoding a functional $\Delta 5$ -fatty acid desaturase. This DNA sequence, encoding a functional $\Delta 5$ -fatty acid desaturase is thought likely to be more closely related to the human $\Delta 5$ -fatty acid desaturase than any of the $\Delta 5$ -fatty acid desaturase gene sequences isolated so far.

[0013] As well as the potential human benefits from the polypeptide encoded by the DNA sequences of this invention, the DNA sequences of this invention may enable the cloning of the equivalent human gene and thereby facilitate overproduction of the human DNA sequence and allow its biomedical exploitation in the treatment of certain human diseases.

[0014] Plant and fungal desaturases are mainly integral membrane polypeptides which makes them difficult to purify and subsequently characterise by conventional methods. Hence, molecular techniques including the use of mutants and transgenic plants have been adopted in order to better study lipid metabolism.

[0015] A first aspect of the invention provides an isolated animal $\Delta 5$ -fatty acid desaturase and functional portions thereof.

[0016] A second aspect of the invention provides an isolated *C. elegans* $\Delta 5$ -fatty acid desaturase.

[0017] A third aspect of the invention provides a DNA sequence according to a first or second aspect of the inven-

tion comprises at least a portion of the sequence shown in SEQ.2 and equivalents to that sequence, or to portions of that sequence, which encode a functional $\Delta 5$ -fatty acid desaturase by virtue of the degeneracy of the genetic code. Preferably, the DNA sequence is derived from a *Caenorhabditis elegans* DNA sequence.

[0018] Preferably, the gene encoding the $\Delta 5$ -fatty acid desaturase encoded by the cloned gene is 1341 bp long. The protein is 447 amino acids long with an estimated molecular weight of 57 kDa.

[0019] Alternatively, the DNA sequence encodes a functional $\Delta 5$ -fatty acid desaturase and comprises at least a portion of the sequence shown in SEQ.1 and equivalents to that sequence, or to portions of that sequence, which encode a functional $\Delta 5$ -fatty acid desaturase by virtue of the degeneracy of the genetic code. Preferably, the DNA sequence is derived from a *Mortierella alpina* DNA sequence.

[0020] Preferably, the gene encoding the $\Delta 5$ -fatty acid desaturase encoded by the cloned gene is 1338 bp long. The protein is 446 amino acids long with an estimated molecular weight of 57 kDa.

[0021] Preferably, a DNA sequence according to a third aspect of the invention is functional in a mammal.

[0022] Preferably, the DNA sequence is expressed in a mammal.

[0023] Preferably, the DNA sequence is expressed in a human.

[0024] Preferably, the DNA sequence is obtained by modification of a functional natural gene encoding a $\Delta 5$ fatty acid desaturase.

[0025] Preferably, the modification includes modification by chemical, physical, or biological means without removing a catalytic activity of the enzyme which it encodes.

[0026] Preferably, the modification improves a catalytic activity of the enzyme which it encodes.

[0027] Preferably, the biological modification includes recombinant DNA methods and forced evolution techniques.

[0028] Preferably, the forced evolution technique is DNA shuffling.

[0029] A fourth aspect of the invention provides a polypeptide encoded by a DNA sequence according to a third aspect of the invention.

[0030] Preferably, at least a portion of the polypeptide has the sequence shown in SEQ.3 or functional equivalents to that sequence or portions of that sequence. Alternatively, at least a portion of the polypeptide has the sequence shown in SEQ.4 or functional equivalents to that sequence or portions of that sequence.

[0031] Preferably, the polypeptide catalyses the conversion of dihomogamma linolenic acid to arachidonic acid.

[0032] Preferably, the polypeptide has been modified without removing the catalytic activity of the encoded polypeptide.

[0033] Preferably, the polypeptide has been modified in such a way as to introduce a specific level of saturation of a substrate at a specific location within the molecular structure of the substrate.

[0034] A fifth aspect of the invention provides a vector containing a DNA sequence of any portion of a DNA sequence according to a third aspect of the invention..

[0035] A sixth aspect of the invention provides a method of producing polyunsaturated fatty acids comprising contacting a substrate with a $\Delta 5$ -fatty acid desaturase according to a first or second aspect of the invention, or a polypeptide according to a fourth aspect of the invention.

[0036] A seventh aspect of the invention provides a method of converting dihomogamma linoleic acid to arachidonic acid wherein the conversion is catalysed by a $\Delta 5$ -fatty acid desaturase according to a first or second aspect of the invention, or a polypeptide according to a fourth aspect of the invention.

[0037] An eighth aspect of the invention provides an organism engineered to produce high levels of a polypeptide according to a fourth aspect of the invention.

[0038] A ninth aspect of the invention provides an organism engineered to produce high levels of a product of a reaction catalysed by a $\Delta 5$ -fatty acid desaturase according to a first or second aspect of the invention, or by a polypeptide according to a fourth aspect of the invention.

[0039] Preferably, the organism has been engineered to carry out the method according to a sixth or seventh aspect of the invention.

[0040] Preferably, the organism is a microorganism.

[0041] Preferably, the microorganism is selected from algae, bacteria and fungi.

[0042] Preferably, the fungi includes phycomycetes. Alternatively, the microorganism is a yeast.

[0043] Alternatively, the organism is a plant. Preferably, the plant is selected from oil seed plants.

[0044] Preferably, the oil seed plants are selected from oil seed rape, sunflower, cereals including maize, tobacco, legumes including peanut and soybean, safflower, oil palm, coconut and other palms, cotton, sesame, mustard, linseed, castor, borage and evening primrose.

[0045] A tenth aspect of the invention provides a seed or other reproductive material derived from an organism according to a ninth aspect of the invention.

[0046] Preferably, the organism is a mammal.

[0047] An eleventh aspect of the invention provides an isolated multienzyme pathway wherein the pathway includes a $\Delta 5$ -fatty acid desaturase according to a first or second aspect of the invention.

[0048] A twelfth aspect of the invention provides a compound produced by a conversion of a substrate, wherein said conversion is catalysed by a $\Delta 5$ -fatty acid desaturase according to a first or second aspect of the invention.

[0049] A thirteenth aspect of the invention provides an intermediate compound produced by the reaction catalysed by a $\Delta 5$ -fatty acid desaturase according to a first or second aspect of the invention.

[0050] A fourteenth aspect of the invention provides a foodstuff or dietary supplement containing a polyunsaturated fatty acid produced by a method according to a sixth aspect of the invention.

[0051] A fifteenth aspect of the invention provides a pharmaceutical preparation containing a polyunsaturated fatty acid produced by a method according to a sixth aspect of the invention.

[0052] A sixteenth aspect of the invention provides prostaglandins synthesised by a biosynthetic pathway including a catalytic activity of a $\Delta 5$ -fatty acid desaturase according to a first or second aspect of the invention.

[0053] A seventeenth aspect of the invention provides a method for the modulation of prostaglandins synthesis by the control of the levels of expression of a DNA sequence according to a third aspect of the invention.

[0054] An eighteenth aspect of the invention provides a probe comprising all or part of a DNA sequence according to a third aspect of the invention, or an equivalent RNA sequence.

[0055] A nineteenth aspect of the invention provides a probe comprising all or part of a $\Delta 5$ -fatty acid desaturase polypeptide according to a fourth aspect of the invention.

[0056] A twentieth aspect of the invention provides a method of isolating $\Delta 5$ -fatty acid desaturases using a probe according to a nineteenth aspect of the invention.

[0057] It is possible that the gene of the invention may be transformed into human cells and exploited in gene therapy techniques at a suitable level in vivo to provide a constant supply of enzyme converting fatty acids to polyunsaturated fatty acids within the patient's body. This could be an effective preventative treatment for example, in patients suffering high levels of cholesterol or other medical conditions where administration of polyunsaturated fatty acids may have beneficial disease-preventative effects.

[0058] In addition, either whole or part of the DNA sequences of the invention, or whole or part of the polypeptide sequences of the invention could be used as search probes for research or diagnostic purposes.

[0059] The invention will now be described by way of example only, with reference to the accompanying drawings, SEQ.1 to SEQ.4, and FIGS. 1 to 4, in which:

[0060] SEQ.1 is a cDNA sequence encoding $\Delta 5$ -fatty acid desaturase from *Mortierella alpina* and;

[0061] SEQ.2 is a cDNA sequence encoding $\Delta 5$ -fatty acid desaturase from *C. elegans*; and

[0062] SEQ.3 is the peptide sequence obtained by translating the gene sequence of SEQ.1; and

[0063] SEQ.4 is the peptide sequence obtained by translating the gene sequence of SEQ.2; and

[0064] FIG. 1 is a line-up of the gene encoding *Mortierella alpina* $\Delta 5$ -fatty acid desaturase with various $\Delta 6$ desaturases and a $\Delta 12$ desaturase; and

[0065] FIG. 2 is a line-up of the gene encoding $\Delta 5$ -fatty acid desaturase with the *C. elegans* $\Delta 6$ desaturase and the fungal $\Delta 5$ desaturase from *M. alpina*; and

[0066] FIG. 3 is a gas chromatography trace of the fatty acid methyl esters of induced yeast cell transformants transformed with the *Mortierella alpina* $\Delta 5$ -fatty acid desaturase gene and uninduced yeast cell transformants; and

[0067] FIG. 4 is a gas chromatography trace of the fatty acid methyl esters of induced yeast cell transformants transformed with the *C. elegans* $\Delta 5$ -fatty acid desaturase gene and uninduced yeast cell transformants.

[0068] Cloning and Sequencing of the $\Delta 5$ -Fatty Acid Desaturase Gene from *Mortierella alpina*

[0069] The DNA sequences of the invention encode $\Delta 5$ -fatty acid desaturases and were cloned using PCR technology in combination with cDNA library templates and specifically designed primers. The function of the DNA sequences, namely the conversion of dihanogamma linolenic acid (DHL) to arachidonic acid (AA), and eicostetraenoate (ETA) to ecosapentaenoate (EPA), were verified by expressing the corresponding cDNAs in yeast.

[0070] The $\Delta 5$ -fatty acid desaturase gene from *Mortierella alpina* was cloned by Polymerase Chain Reaction (PCR) techniques using cDNA from *Mortierella alpina* as the template and specifically designed degenerate oligonucleotide primers (DP) as shown below, based on the first and third histidine bases of plant $\Delta 12$ and $\Delta 15$ desaturases previously identified by Shanklin (Shanklin, J, Whittle, E J & Fox, B G. *Biochemistry*. 33, 12787-12794 (1994)). ps Degenerate Oligonucleotide Primers (DP)

5' -GCGAATTA(A/T)TIGGICA(T/C)GA(T/C)TG(T/C)GICA-3'

5' -GCGAATTCATIT(G/T)IGG(A/G)AAIA(G/A)(A/G)TG(A/G)TG-3'

[0071] where I represents inosine, and the Eco RI sites are underlined.

[0072] The PCR amplifications were run entirely conventionally on a thermal cycler made by using a program of 2 minutes at 94° C. then 45 seconds at 94° C., 1 minute at 55° C. and 1 minute at 72° C. for 32 cycles followed by extension at 72° C. for a further 10 minutes. PCR amplification products were separated on 1% agarose gels.

[0073] The range of PCR products amplified from the *Mortierella alpina* cDNA template included a 660 bp product which was gel purified, cloned into pGEM-T (Promega®) and transformed into the *Escherichia coli* expression host, DH5 α .

[0074] Primers (P) were designed against the 660 bp product sequence and fragment amplification carried out by PCR using the cloned 660 bp fragment as a template, and sequence-specific primers (P) based on the 660 bp product sequence.

Delta B for 5' -GATGCGTCTCACTTTTCA-3'

Delta B rev. 5' -GTGGTGACAGCCTGGTAGTT-3'

[0075] The products of this PCR amplification were gel purified and used as probes to screen a *Mortierella alpina* cDNA library. The fragment probe hybridised to 25 out of the 3.5 $\times 10^5$ phage clones screened and one clone was shown, by restriction analysis, to have the expected size of 1.5 kb. This clone, designated L11, was selected for further analysis.

[0076] Sequence analysis of L11 revealed an open reading frame of 1,338 bp in length encoding a polypeptide of 446

amino acids. When analyzed on the protein and genomic databases using the GCG 8 Program (Devereux J. et al. *Nucleic Acids. Res.* 12, 387-395 (1984)), L11 showed a low level 20% identity to the $\Delta 6$ desaturase gene from *Synechocystis* sp. PCC6803 (FIG. 1).

[0077] In FIG. 1, the sequences in the line-up have the following Accession numbers:

S54259	$\Delta 12$ Spirulina	Accession number: X86736
S54809	$\Delta 6$ Spirulina	Accession number: X87094
S68358	Putative Sphingolipid desaturase	Accession number: X87143
S35157	$\Delta 6$ <i>Synechocystis</i>	Accession number: L11421
PBOR6	$\Delta 6$ Borage	Accession number U79010
FU2	$\Delta 5$ desaturase	Accession number AF054824

[0078] In addition, although all three histidine boxes characteristic of desaturase enzymes are present in the translated sequence, the third histidine box located at position 1159 bp in the sequence contains the variant QXXHH. The translated sequence also contains a cytochrome b_5 -like heme-binding domain at the N-terminus which includes the EHPGG motif whereas previously, this feature has only been observed at the C-terminus of other fungal desaturases.

[0079] Southern Blotting of Genomic DNA

[0080] Sequence specific primers (P) designed against the L11 sequence between histidine boxes 1 and 3 of L11, were used in a PCR reaction to amplify a 660 bp region of the L11 sequence.

[0081] The 660 bp PCR product was gel purified and Southern blots of restricted *Mortierella alpina* and *Mucor circinelloides* genomic DNA carried out using the 660 bp fragment as a probe. The results suggest that the gene encoding the $\Delta 5$ -fatty acid desaturase of the invention is present in single copy in *Mortierella alpina* and appears to be absent from *Mucor circinelloides*. In addition, there is no detectable $\Delta 5$ -fatty acid desaturase activity in *Mucor circinelloides*.

[0082] Expression of the Cloned *Mortierella alpina* Gene Encoding $\Delta 5$ -Fatty Acid Desaturase

[0083] In order to confirm that the L11 sequence encoded a $\Delta 5$ -fatty acid desaturase enzyme, the cDNA was subcloned into the yeast expression vector, pYES2, supplied by Invitrogen™ under the control of the GAL4 polymerase promoter to yield plasmid pYES2/L11. The expression of L11 was checked by in vitro transcription-translation of pYES2/L11 using the Promega™ coupled Transcription and Translation system. ^{35}S methionine-labelled translation products were generated which were run on SDS PAGE and visualised by exposure to autoradiograph film. The estimated molecular weight of the product was 55-60 kD and a control plasmid, pYES2 with no insert, failed to yield any labelled translation product.

[0084] Construct, pYES2/L11, was transformed into yeast *Saccharomyces cerevisiae* and grown on uracil-deficient YCA medium. Transformants were selected by virtue of the presence of the URA3 selectable marker carried by pYES2/L11 and expression of L11 was induced by the addition of galactose to a final concentration of 1% mM. The cultures were grown overnight in the presence of 0.5 mM dihomo gamma linolenate, detergent (1% tergitol NP-40) and 2% raffinose. Aliquots were harvested at t=0, t=4 hours, and t=16 hours.

[0085] Yeast total fatty acids were analysed by GC of methyl esters. The lipids from the induced and uninduced control samples were transmethylated with 1M HCL in methanol at 80° C. for 1 hr. Fatty acid methyl esters (FAMES) were extracted in hexane. GC analysis of FAMES was conducted using a Hewlett Packard 58804 Series Gas Chromatograph equipped with a 25M \times 0.32 mm RSL-500 BP bonded capillary column and a flame ionization detector.

[0086] When methyl esters of the total fatty acids isolated from yeast carrying the plasmid pYes2/L11 and grown in the presence of galactose and dihomo gamma linolenic acid were analysed by GC an additional peak was observed (see FIG. 3). This extra peak had the same retention time as the authentic arachidonic acid standard (Sigma) indicating that the transgenic yeast were capable of desaturating Dihomo gamma linolenic acid at the $\Delta 5$ position. No such peaks were observed in any of the control samples (transformation with pYes2) FIG. 3. The identity of the additional peak was confirmed by GCMS (Kratos MS80RFA operating at an ionization voltage of 70 eV with a scan range of 500-40 daltons) which positively identified this compound as arachidonic acid.

[0087] This demonstrates that the DNA sequence from *Mortierella alpina* encodes a functional polypeptide involved in the synthesis of arachidonic acid in the presence of galactose and dihomo gamma linolenate.

[0088] Cloning and Sequencing of the *C. elegans* $\Delta 5$ -Fatty Acid Desaturase Gene

[0089] Previously, the inventors identified fungal $\Delta 5$ and $\Delta 6$ -fatty acid desaturases from both plant and animal species which were distinct from previously identified microsomal desaturases. This difference was due to the presence of an N-terminal extension which showed homology to the electron donor protein cytochrome b_5 .

[0090] During the characterisation of the fungal (*Mortierella alpina*) $\Delta 5$ -fatty acid desaturase and the *C. elegans* $\Delta 6$ -fatty acid desaturase (present on cosmid W08D2 (Accession No. Z70271)), the inventors identified a related sequence on cosmid T13F2.1 (Accession No. Z81122) also containing *C. elegans* DNA likely to encode a fatty acid desaturase.

[0091] Analysis of the sequences (using Genefinder program (Wilson, R. et al (1994) *Nature*, 368, 32-38)) revealed that cosmids W08D2 and T13F2 contained overlapping regions. In addition, it was found that cosmid T13F2 contained an open reading frame (ORF), designated T13F2.1, which contained an N-terminal cytochrome b_5 domain (defined by the diagnostic His-Pro-Gly-Gly motif), as well as three 'histidine boxes' characteristic of all microsomal desaturases. Further, this putative desaturase contained a variant third histidine box, with a H \rightarrow Q substitution for the first histidine in the His-X-X-His-His motif. This glutamate substitution is present in both plant and animal $\Delta 6$ -fatty acid desaturases and in the fungal $\Delta 5$ -fatty acid desaturase from *M. alpina*.

[0092] The overlap between cosmids T13F2 and W08D2 allowed the determination of the proximity of the putative desaturase ORF, T13F2.1, to the $\Delta 6$ -fatty acid desaturase, revealing that the two sequences were arranged in tandem on chromosome IV, separated by 990 bases from the predicted stop codon of T13F2.1 to the initiating methionine triplet of the $\Delta 6$ -fatty acid desaturase.

[0093] Since sequence analysis predicted that the T13F2.1 ORF was interspersed with a number of introns, heterolo-

gous functional expression of genomic DNA was unfeasible. Therefore, the polymerase chain reaction (PCR) was used to amplify a partial cDNA clone corresponding to a large predicted exon at the 5' end of the T13F2.1 ORF using the following primers, CEFOR AND CEREV:

CEFOR: 5' - ATGGTATTACGAGACGAAGA - 3'

CEREV: 5' - TCTGGGATCTCTGGTTCTTG - 3'

[0094] After initial denaturation at 94° C. for 2 minutes, amplification was performed in 32 cycles of: 45 seconds at 94° C., 1 minute at 55° C., and 1 minute at 72° C. followed by a final extension at 72° C. for a further 10 minutes.

[0095] A DNA fragment of the correct predicted size was amplified (as visualised on a 1% agarose gel), the gel band was cut out, the DNA purified and ligated directly into pGEM-T (Promega), and the resulting plasmid transformed into *E. coli* DH5 α cells. Plasmid DNA was purified for sequencing using the Qiagen QIAprep miniprep kit, and the nucleotide sequence of the insert determined by automated sequencing using an ABI-377 DNA sequencer.

[0096] In order to isolate the complete coding region corresponding to ORF T13F2.1, this isolated 233 bp PCR-amplified fragment was used to screen a mixed stage *C. elegans* cDNA library that had been constructed in λ ZapII by Prof Yuji Kohara—Mishima, Japan. The screening was carried out using standard techniques (Sambrook et al (1989) Molecular Cloning. A Laboratory Manual) using the cloned PCR product as a probe. The DNA fragments were labelled with α [³²P] d CTP using the Ready to Go DNA-Labeling reaction mix (Pharmacia). Of 1.4 \times 10⁵ pfu screened for hybridization to the 233 bp fragment, 5 plaques gave positive signals and were cored out of the agar plates and eluted into SM buffer. The resultant phage suspensions were screened for the presence of T13F2.1 by PCR amplification using CEFOR and CEREV. One clone, designated L4, was purified by 2 additional rounds of plating and hybridisation screening at 65° C. using the 233 bp fragment isolated by PCR. Plasmid L4 was released from λ clone L4 by excision and the cDNA insert sequenced on both strands using a Perkin Elmer AB1-377 DNA sequencer.

[0097] The resulting DNA sequence is shown in SEQ.2, and the predicted amino acid sequence is shown in SEQ.4.

[0098] Functional Analysis of L4 in Yeast

[0099] The complete coding region (coding for 447 amino acids) of L4 was amplified by PCR using the primers YCEDFor and TCEDRev shown below, which also introduced flanking HindIII and BamHI restriction sites:

[0100] YCEDFor:

[0101] 5'-GCGAAGCTTAAAATGGTATTACGAGAG-CAAGAGC-3'

[0102] (annealing to the initiating methionine is indicated by the bold type face and the Hind III restriction site is underlined)

[0103] YCEDRev:

[0104] 5'-GCGGATCCAATCTAGGCAATCTTTTAGT-CAA-3'

[0105] The amplified PCR product containing the complete coding region of L4 was ligated into the yeast expression vector, pYES2 (Invitrogen), downstream of the GAL1

promoter using HindIII and BamIII restriction sites (enzymes supplied by Boehringer Mannheim). The resulting construct, designated pYES2/L4, was transformed into *E. coli*, and the fidelity of the PCR-generated insert in plasmid pYES2/L4 was confirmed in vitro by coupled transcription/translation using the TNT system (Promega). The resulting translation products were labelled with ³⁵S methionine, separated by SDS-PAGE and visualised by autoradiography.

[0106] The translation product obtained from pYES2/L4 had a molecular weight of approximately M_r57,000, whereas the control vector, pYES2 with no insert, did not yield a translation product.

[0107] For functional analysis of the L4 coding region the recombinant plasmid was transformed into *S. cerevisiae* DBY746 by the lithium acetate method (Elble R. (1992) Bio Techniques 13 18-20). Cells were cultured overnight in a medium containing raffinose as a carbon source, and supplemented by the addition of either linoleic acid (18:2 $\Delta^{9,12}$) or di-homo- γ -linolenic acid (C20:3 $\Delta^{8,11,14}$) in the presence of 1% tergitol (as described by Napier et al (1998) Biochem. J. 330 611-614). These fatty acids are not present in *S. cerevisiae* but serve as the specific substrates for either the Δ^6 or Δ^5 -desaturase, respectively. Expression of the L4 coding region from the GAL1 promoter of the vector was induced by the addition of galactose to 1%. Growth of the cultures was continued for 16 hours before removal of aliquots for the analysis of fatty acids by GC Total fatty acids extracted from yeast cultures were analysed by gas chromatography (GC) of methyl esters. Lipids were transmethylated with 1M HCl in methanol at 80° C. for 1 hr, then fatty acid methyl esters (FAMES) were extracted in hexane. GC analysis of FAMES were conducted using a Hewlett Packard 5880A Series Gas chromatograph equipped with a 25 M \times 0.32 mm RSL-500 BP bonded capillary column and a flame ionization detector. Fatty acids were identified by comparison with retention times of FAME standards (Sigma). Relative percentages of the fatty acids were estimated from peak areas. Arachidonic acid was identified by GC-MS using a Krats MS80RFA operating at an ionization voltage of 70 eV, with a scan range of 500-40 daltons. FIG. 4 shows the result of GC analysis of the fatty acid methyl esters of transformed yeast strains. An additional peak is apparent in the trace obtained from induced pYES2/L4 grown in the presence of di-homo- γ -linolenic acid compared to an empty-vector control. This peak was also absent from uninduced cultures grown on di-homo- γ -linolenic acid and it is also important to note that pYES2/L4 grown in the presence of linoleic acid failed to accumulate any novel peaks indicating that this fatty acid is not a substrate for the enzyme encoded by the *C. elegans* cDNA. The retention time of the additional peak is identical to that of the authentic methyl-arachidonic acid standard. The fatty acid produced from di-homo- γ -linolenic acid was further characterised by GCMS (Gas Chromatography Mass Spectrometry) and identified as arachidonic acid. The results show, therefore, that yeast cells transformed with the plasmid pYES2/L4 had acquired functional Δ^5 -desaturase activity and were now capable of synthesising arachidonic acid from the substrate di-homo- γ -linolenic acid. The Δ^5 -desaturase in the transformed yeast appeared to be an efficient catalyst.

[0108] This demonstrates that the DNA sequence from *C. elegans* encodes a functional polypeptide involved in the synthesis of arachidonic acid in the presence of galactose and di-homo- γ -linolenate.

```

1 ATGGTATTAC GAGAGCAAGA GCATGAGCCA TTCTTCATTA AAATTGATGG SEQ. 2
51 AAAATGGTGT CAAATTGACG ATGCTGTCCT GAGATCACAT CCAGGTGGTA
101 GTGCAATTAC TACCTATAAA AATATGGATG CCACTACCGT ATTCCACACA
151 TTCCATACTG GTTCTAAAGA AGCGTATCAA TGGCTGACAG AATTGAAAAA
201 AGAGTGCCCT ACACAAGAAC CAGAGATCCC AGATATTAAG GATGACCCAA
251 TCAAAGGAAT TGATGATGTG AACATGGGAA CTTTCAATAT TTCTGAGAAA
301 CGATCTGCCC AAATAAATAA AAGTTTCACT GATCTACGTA TGCAGTTCG
351 TGCAGAAGGA CTTATGGATG GATCTCCTTT GTTCTACATT AGAAAAATTC
401 TTGAAACAAT CTTCACAATT CTTTTTGCAT TCTACCTCA ATACCACACA
451 TATTATCTTC CATCAGCTAT TCTAATGGGA GTTGCCTGGC AACAAATGGG
501 ATGGTTAATC CATGAATTCG CACATCATCA GTTGTTCAA AACAGATACT
551 ACAATGATTT GGCCAGCTAT TTCGTTGGAA ACTTTTACA AGGATTCTCA
601 TCTGGTGGTT GAAAAGAGCA GCACAATGTG CATCACGCAG CCACAAATGT
651 TGTTGGACGA GACGGAGATC TTGATTTAGT CCCATTCTAT GCTACAGTGG
701 CAGAACATCT CAACAATTAT TCTCAGGATT CATGGGTAT GACTCTATTC
751 AGATGGCAAC ATGTTTCATTG GACATTCATG TTACCATTCC TCCGTCTCTC
801 GTGGCTTCTT CAGTCAATCA TTTTGTGTAG TCAGATGCCA ACTCATTATT
851 ATGACTATTA CAGAAATACT GCGATTTATG AACAGGTTGG TCTCTCTTTG
901 CACTGGGCTT GGTCAATTGGG TCAATTGTAT TTCCTACCCG ATTGGTCAAC
951 TAGAATAATG TTCTTCCTTG TTTCTCATCT TGTTGGAGGT TTCCTGCTCT
1001 CTCATGTAGT TACTTTCAAT CATTATTCAG TGGAGAAGTT TGCATTGAGC
1051 TCGAACATCA TGTCAAATTA CGCTTGTCTT CAAATCATGA CCACAAGAAA
1101 TATGAGACCT GGAAGATTCA TTGACTGGCT TTGGGGAGGT CTTAACTATC
1151 AGATTGAGCA CCATCTTTTC CCAACGATGC CACGACACAA CTTGAACACT
1201 GTTATGCCAC TTGTTAAGGA GTTTCAGCA GCAAATGGTT TACCATACAT
1251 GGTCGACGAT TATTTCACAG GATTCTGGCT TGAAATTGAG CAATCCGAA
1301 ATATTGCAAA TGTGCTGCT AAATTGACTA AAAAGATTGC CTAG

```

```

1 MGTDQKFTT WEELAAHNTK GDLFLAIRGR VYDVKFLSR HPGGVDLLLL SEQ. 3
51 GAGRDVTPVF EMYHAFGAAD AIMKKYYVGT LVSNELPVFP EPTVFHKTIK
101 TRVEGYFTDR DIDPKNRPEI WGRYALIFGS LIASYAQLF VPFVVERTWL
151 QVVFIIIMGF ACAQVGLNPL HDASHFSVTH NPTVWKILGA THDFFNGASY
201 LVWMYQHMLG HHPYTNIAGA DPDVSTFEPD VVRIKPNQKW FVNHNQDMF
251 VPFLYGLLAF KVRIQDINIL YFVKTNDAIR VNPISTWHTV MFWGGKAFFV
301 WYRLIVPLQY LPLGKVLFFF TVADMVSSYW LALTFQANH VEEVQWPLPD
351 ENGIQKQDWA AMQVETTQDY AHDShLWTSI TGSlnYQAVH HLFpNVsQHH
401 YPDILAIKN TCSEYKVPYL VKDTFWQAFa SHLEHLRVLG LRPKEE*

```

The predicted amino acid sequence of L4 the gene which encodes a Δ^5 fatty acid desaturase from *C. elegans*.

```

1 MVLREQEHEP FFIKIDGKWC QIDDAVLRSH PGGSaITTYK NMDATTVFHT SEQ 4

```

-continued

51 FHTGSKEAYQ WLTELKKECP TQEPFIPDIK DDPIKGIDDV NMGTFNISEK
 101 RSAQINKSFT DLRMRVRAEG LMDGSPLFYI RKILETIFTI LFAFYLYQYHT
 151 YYLPSAILMG VAWQQLGWLI HEFAHHQLFK NRYYNDLASY FVGNFLQGFS
 201 SGGWKEQHNH HHAATNVVGR DGDLDLVFPY ATVAEHLNNY SQDSWVMTLF
 251 RWQHVHWTM LPFLRLSWLL QSIIFVSQMP THYYDYRNT AIYEQVGLSL
 301 HWAWSLQLY FLPDWSTRIM FFLVSHLVGG FLLSHVVTFN HYSVEKFALS
 351 SNIMSNYACL QIMTTRNMRP GRFIDWLWGG LNYQIEHHP PTMPRHNLNT
 401 VMPLVKEFAA ANGLPYMVDD YFTGFWLEIE QFRNIANVAA KLTKKIA*

[0109]

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 23

<210> SEQ ID NO 1

<211> LENGTH: 1405

<212> TYPE: DNA

<213> ORGANISM: Mortierella alpina

<400> SEQUENCE: 1

atgggtacgg accaaggaaa aaccttcacc tgggaagagc tagcggccca taacaccaag	60
ggcgaccttt ttttgccat ccgcggcagg gtgtacgatg tcacaaagtt cttgagccgc	120
catcctggtg gagtggacac tctcctgctc ggagctggcc gagatgttac tccggtcttt	180
gagatgtatc acgcgtttgg ggctgcagat gccatcatga agaagtacta tgcggtaca	240
ttggtttcga atgagctgcc cgtcttcccg gagccaacgg tgttccacaa aaccatcaag	300
acgagagttg agggctactt tacggatcgg gacattgatc ccaagaacag accagagatc	360
tggggacgat acgctcttat ctttgatcc ttgatcgctt cctactacgc gcagctcttt	420
gtgcctttcg ttgtcgaacg cacatggctc caggtgggtg ttgcaatcat catgggattt	480
gcgtgcgcac aagtcggact caaccctctt catgatgcgt ctcacttttc agtgaccac	540
aacccactg tctggaagat tctgggagcc acgcacgact ttttcaacgg agcatcgtac	600
ctgggtgga tgtaccaaca tatgctcggc catcacccct acaccaacat tgctggagca	660
gatactgacg tgtcgacgtt tgagcccgat gttcgtcgta tcaagccaa ccaaaagtgg	720
tttgtaacc acatcaacca ggacatgtt gttcctttcc tgtacggact gctggcgctc	780
aaggtgcgca ttcaggacat caacattttg tactttgtca agacaaatga cgcaattcgc	840
gtcaatccca tctcgacatg gcacactgtg atgttctggg gcggcaaggc tttctttgtc	900
tggtatcgcc tgattgttcc cctgcagtat ctgcccttg gcaagtgct gtcctgttc	960
acggtcgagg acatggtgtc gtcttactgg ctggcgctga ccttccaggc gaaccacgtt	1020
gttgaggaag ttcagtggcc gttgcctgac gagaacggga tcatccaaaa ggactgggca	1080
gctatgcagg ttgagactac gcaggattac gcacacgact cgcacctctg gaccagcatt	1140
actggcagct tgaactacca ggctgtgcac catctgttcc ccaacgtgtc gcagcaccat	1200
tatcccagata ttctggccat catcaagaac acctgcagcg agtacaaggt tccatacctt	1260

-continued

```
gtcaaggata ccttttggca agcatttgct tcacatttgg agcaattgcg tgttcttgga 1320
ctccgtccca aggaagagta gaaagaaaaa aagcgccgaa cgaagtattg cccctttttt 1380
ctccaagaaa aaaaaaaaaa aaaaaa 1405
```

```
<210> SEQ ID NO 2
<211> LENGTH: 1344
<212> TYPE: DNA
<213> ORGANISM: C. elegans
```

```
<400> SEQUENCE: 2
```

```
atggtattac gagagcaaga gcatgagcca ttcttcatta aaattgatgg aaaatggtgt 60
caaattgacg atgctgtcct gagatcacat ccagggtgga gtgcaattac tacctataaa 120
aatatggatg ccactaccgt attccacaca ttccatactg gttctaaaga agcgtatcaa 180
tggctgacag aattgaaaaa agagtgcctt acacaagaac cagagatccc agatattaag 240
gatgacccaa tcaaaggaat tgatgatgtg aacatgggaa ctttcaatat ttctgagaaa 300
cgatctgccc aaataaataa aagtttcact gatctacgta tgcgagttcg tgcagaagga 360
cttatggatg gatctccttt gttctacatt agaaaaatto ttgaaacaat cttcacaatt 420
ctttttgcat tctaccttca ataccacaca tattatcttc catcagctat tctaattgga 480
gttgctgtggc acaaatggg atgggtaatc catgaattcg cacatcatca gttgttcaaa 540
aacagatact acaatgattt ggccagctat ttctgtgga actttttaca aggattctca 600
tctggtggtt ggaagagca gcacaatgtg catcacgcag ccacaaatgt tgttgacga 660
gacggagatc ttgatttagt cccattctat gctacagtgg cagaacatct caacaattat 720
tctcaggatt catgggttat gactctatc agatggcaac atgttcattg gacattcatg 780
ttaccattcc tccgtctctc gtggcttctt cagtcaatca tttttgtag tcagatgcca 840
actcattatt atgactatta cagaaatact gcgatttatg aacagggttg tctctctttg 900
cactgggctt ggtcattggg tcaattgtat ttctaccg attggtcaac tagaataatg 960
ttcttccttg tttctcatct tgttgagggt ttctgtctct ctcatgtagt tactttcaat 1020
cattattcag tggagaagtt tgcattgagc tcgaacatca tgtcaaatta cgctgtgctt 1080
caaatcatga ccacaagaaa tatgagacct ggaagattca ttgactggct ttggggaggt 1140
cttaactatc agattgagca ccatcttttc ccaacgatgc cagcacacaa cttgaacact 1200
gttatgccac ttgttaagga gtttgagca gcaaatggtt taccatacat ggtcgacgat 1260
tatttcacag gattctggct tgaaattgag caattccgaa atattgcaa tgttgctgct 1320
aaattgacta aaaagattgc ctag 1344
```

```
<210> SEQ ID NO 3
<211> LENGTH: 446
<212> TYPE: PRT
<213> ORGANISM: Mortierella alpina
```

```
<400> SEQUENCE: 3
```

```
Met Gly Thr Asp Gln Gly Lys Thr Phe Thr Trp Glu Glu Leu Ala Ala
  1             5             10             15
His Asn Thr Lys Gly Asp Leu Phe Leu Ala Ile Arg Gly Arg Val Tyr
          20             25             30
Asp Val Thr Lys Phe Leu Ser Arg His Pro Gly Gly Val Asp Thr Leu
          35             40             45
```

-continued

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

-continued

```

<210> SEQ ID NO 4
<211> LENGTH: 448
<212> TYPE: PRT
<213> ORGANISM: C. elegans

<400> SEQUENCE: 4
Met Val Leu Arg Glu Gln Glu His Glu Pro Phe Phe Ile Lys Ile Asp
 1          5          10          15
Gly Lys Trp Cys Gln Ile Asp Asp Ala Val Leu Arg Ser His Pro Gly
 20          25          30
Gly Ser Ala Ile Thr Thr Tyr Lys Asn Met Asp Ala Thr Thr Val Phe
 35          40          45
His Thr Phe His Thr Gly Ser Lys Glu Ala Tyr Gln Trp Leu Thr Glu
 50          55          60
Lys Leu Lys Lys Glu Cys Pro Thr Gln Glu Pro Glu Ile Pro Asp Ile
 65          70          75          80
Lys Asp Asp Pro Ile Lys Gly Ile Asp Asp Val Asn Met Gly Thr Phe
 85          90          95
Asn Ile Ser Glu Lys Arg Ser Ala Gln Ile Asn Lys Ser Phe Thr Asp
100          105          110
Leu Arg Met Arg Val Arg Ala Glu Gly Leu Met Gly Asp Ser Pro Leu
115          120          125
Phe Tyr Ile Arg Lys Ile Leu Glu Thr Ile Phe Thr Ile Leu Phe Ala
130          135          140
Phe Tyr Leu Gln Tyr His Thr Tyr Tyr Leu Pro Ser Ala Ile Leu Met
145          150          155          160
Gly Val Ala Trp Gln Gln Leu Gly Trp Leu Ile His Glu Phe Ala His
165          170          175
His Gln Leu Phe Lys Asn Arg Tyr Tyr Asn Asp Leu Ala Ser Tyr Phe
180          185          190
Val Gly Asn Phe Leu Gln Gly Phe Ser Ser Gly Gly Trp Lys Glu Gln
195          200          205
His Asn Val His His Ala Ala Thr Asn Val Val Gly Arg Asp Gly Asp
210          215          220
Leu Asp Leu Val Pro Phe Tyr Ala Thr Val Ala Glu His Leu Asn Asn
225          230          235          240
Tyr Ser Gln Asp Ser Trp Val Met Thr Leu Phe Arg Trp Gln His Val
245          250          255
His Trp Thr Phe Met Leu Pro Phe Leu Arg Leu Ser Trp Leu Leu Gln
260          265          270
Ser Ile Ile Phe Val Ser Gln Met Pro Thr His Tyr Tyr Asp Tyr Tyr
275          280          285
Arg Asn Thr Ala Ile Tyr Glu Gln Val Gly Leu Ser Leu His Trp Ala
290          295          300
Trp Ser Leu Gly Gln Leu Tyr Phe Leu Pro Asp Trp Ser Thr Arg Ile
305          310          315          320
Met Phe Phe Leu Val Ser His Leu Val Gly Gly Phe Leu Leu Ser His
325          330          335
Val Val Thr Phe Asn His Tyr Ser Val Glu Lys Phe Ala Leu Ser Ser
340          345          350
Asn Ile Met Ser Asn Tyr Ala Cys Leu Gln Ile Met Thr Thr Arg Asn
355          360          365

```

-continued

Met Arg Pro Gly Arg Phe Ile Asp Trp Leu Trp Gly Gly Leu Asn Tyr
 370 375 380

Gln Ile Glu His His Leu Phe Pro Thr Met Pro Arg His Asn Leu Asn
 385 390 395 400

Thr Val Met Pro Leu Tyr Lys Glu Phe Ala Ala Ala Asn Gly Leu Pro
 405 410 415

Tyr Met Val Asp Asp Tyr Phe Thr Gly Phe Trp Leu Glu Ile Glu Gln
 420 425 430

Phe Arg Asn Ile Ala Asn Val Ala Ala Lys Leu Thr Lys Lys Ile Ala
 435 440 445

<210> SEQ ID NO 5
 <211> LENGTH: 24
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 5

gcgaattawt ggcaygaytg ygca

24

<210> SEQ ID NO 6
 <211> LENGTH: 25
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 6

gcgaattcat tkggraaarr tgrtg

25

<210> SEQ ID NO 7
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 7

gatgcgtctc acttttca

18

<210> SEQ ID NO 8
 <211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 8

gtgggtgcaca gcctggtagt t

21

<210> SEQ ID NO 9
 <211> LENGTH: 351
 <212> TYPE: PRT
 <213> ORGANISM: Spirulina

<400> SEQUENCE: 9

Met Thr Leu Ser Ile Val Lys Ser Glu Asp Ser Ser Ser Arg Pro Ser
 1 5 10 15

Ala Val Pro Ser Asp Leu Pro Leu Glu Glu Asp Ile Ile Asn Thr Leu

-continued

20					25					30					
Pro	Ser	Gly	Val	Phe	Val	Gln	Asp	Arg	Tyr	Lys	Ala	Trp	Met	Thr	Val
		35					40					45			
Ile	Ile	Asn	Val	Val	Met	Val	Gly	Leu	Gly	Trp	Leu	Gly	Ile	Ala	Ile
		50					55					60			
Ala	Pro	Trp	Phe	Leu	Leu	Pro	Val	Val	Trp	Val	Phe	Thr	Gly	Thr	Ala
65							70					75			80
Leu	Thr	Gly	Phe	Phe	Val	Ile	Gly	His	Asp	Cys	Gly	His	Arg	Ser	Phe
			85						90					95	
Ser	Arg	Asn	Val	Trp	Val	Asn	Asp	Trp	Val	Gly	His	Ile	Leu	Phe	Leu
			100					105					110		
Pro	Ile	Ile	Tyr	Pro	Phe	His	Ser	Trp	Arg	Ile	Gly	His	Asn	Gln	His
			115					120					125		
His	Lys	Tyr	Thr	Asn	Arg	Met	Glu	Leu	Asp	Asn	Ala	Trp	Gln	Pro	Trp
			130					135					140		
Arg	Lys	Glu	Glu	Tyr	Gln	Asn	Ala	Gly	Lys	Phe	Met	Gln	Val	Thr	Tyr
145							150					155			160
Asp	Leu	Phe	Arg	Gly	Arg	Ala	Trp	Trp	Ile	Gly	Ser	Ile	Leu	His	Trp
				165										175	
Ala	Ser	Ile	His	Phe	Asp	Trp	Thr	Lys	Phe	Glu	Gly	Lys	Gln	Arg	Gln
			180						185					190	
Gln	Val	Lys	Phe	Ser	Ser	Leu	Leu	Val	Ile	Gly	Ala	Ala	Ala	Ile	Ala
			195					200					205		
Phe	Pro	Thr	Met	Ile	Leu	Thr	Ile	Gly	Val	Trp	Gly	Phe	Val	Lys	Phe
			210					215					220		
Trp	Val	Ile	Pro	Trp	Leu	Val	Phe	His	Phe	Trp	Met	Ser	Thr	Phe	Thr
225							230					235			240
Leu	Leu	His	His	Thr	Ile	Ala	Asp	Ile	Pro	Phe	Arg	Glu	Pro	Glu	Gln
				245					250					255	
Trp	His	Glu	Ala	Glu	Ser	Gln	Leu	Ser	Gly	Thr	Val	His	Cys	Asn	Tyr
			260						265					270	
Ser	Arg	Trp	Gly	Glu	Phe	Leu	Cys	His	Asp	Ile	Asn	Val	His	Ile	Pro
			275					280					285		
His	His	Val	Thr	Thr	Ala	Ile	Pro	Trp	Tyr	Asn	Leu	Arg	Thr	Pro	Thr
			290					295					300		
Pro	Val	Tyr	Arg	Lys	Ile	Gly	Gly	Glu	Tyr	Leu	Tyr	Pro	Glu	Cys	Asp
305								310				315			320
Phe	Ser	Trp	Gly	Leu	Met	Lys	Gln	Val	Val	Asp	His	Ala	Ile	Cys	Met
				325					330					335	
Met	Arg	Ile	Thr	Ile	Ile	Ser	Gln	Ser	Leu	Thr	Thr	Lys	Arg	Val	
			340					345					350		
<210> SEQ ID NO 10															
<211> LENGTH: 368															
<212> TYPE: PRT															
<213> ORGANISM: Spirulina															
<400> SEQUENCE: 10															
Met	Thr	Ser	Thr	Thr	Ser	Lys	Val	Thr	Phe	Gly	Lys	Ser	Ile	Gly	Phe
1				5					10					15	
Arg	Lys	Glu	Leu	Asn	Arg	Arg	Val	Asn	Ala	Tyr	Leu	Glu	Ala	Glu	Asn
			20					25					30		

-continued

Ile	Ser	Pro	Arg	Asp	Asn	Pro	Pro	Met	Tyr	Leu	Lys	Thr	Ala	Ile	Ile
35						40						45			
Leu	Ala	Trp	Val	Val	Ser	Ala	Trp	Thr	Phe	Val	Val	Phe	Gly	Pro	Asp
50						55				60					
Val	Leu	Trp	Met	Lys	Leu	Leu	Gly	Cys	Ile	Val	Leu	Gly	Phe	Gly	Val
65				70				75						80	
Ser	Ala	Val	Gly	Phe	Asn	Ile	Ser	His	Asp	Gly	Asn	His	Gly	Gly	Tyr
				85				90						95	
Ser	Lys	Tyr	Gln	Trp	Val	Asn	Tyr	Leu	Ser	Gly	Leu	Thr	His	Asp	Ala
		100				105						110			
Ile	Gly	Val	Ser	Ser	Tyr	Leu	Trp	Lys	Phe	Arg	His	Asn	Val	Leu	His
		115				120						125			
His	Thr	Tyr	Thr	Asn	Ile	Leu	Gly	His	Asp	Val	Glu	Ile	His	Gly	Asp
130						135				140					
Glu	Leu	Val	Arg	Met	Ser	Pro	Ser	Met	Glu	Tyr	Arg	Trp	Tyr	His	Arg
145				150						155				160	
Tyr	Gln	His	Trp	Phe	Ile	Trp	Phe	Val	Tyr	Pro	Phe	Ile	Pro	Tyr	Tyr
				165				170						175	
Trp	Ser	Ile	Ala	Asp	Val	Gln	Thr	Met	Leu	Phe	Lys	Arg	Gln	Tyr	His
		180						185				190			
Asp	His	Glu	Ile	Pro	Ser	Pro	Thr	Trp	Val	Asp	Ile	Ala	Thr	Leu	Leu
		195				200						205			
Ala	Phe	Lys	Ala	Phe	Gly	Val	Ala	Val	Phe	Leu	Ile	Ile	Pro	Ile	Ala
210						215				220					
Val	Gly	Tyr	Ser	Pro	Leu	Glu	Ala	Val	Ile	Gly	Ala	Ser	Ile	Val	Tyr
225				230						235				240	
Met	Thr	His	Gly	Leu	Val	Ala	Cys	Val	Val	Phe	Met	Leu	Ala	His	Val
				245				250						255	
Ile	Glu	Pro	Ala	Glu	Phe	Leu	Asp	Pro	Asp	Asn	Leu	His	Ile	Asp	Asp
		260						265				270			
Glu	Trp	Ala	Ile	Ala	Gln	Val	Lys	Thr	Thr	Val	Asp	Phe	Ala	Pro	Asn
		275				280						285			
Asn	Pro	Ile	Ile	Asn	Trp	Tyr	Val	Gly	Gly	Leu	Asn	Tyr	Gln	Thr	Val
290						295				300					
His	His	Leu	Phe	Pro	His	Ile	Cys	His	Ile	His	Tyr	Pro	Lys	Ile	Ala
305				310						315				320	
Pro	Ile	Leu	Ala	Glu	Val	Cys	Glu	Glu	Phe	Gly	Val	Asn	Tyr	Ala	Val
				325				330						335	
His	Gln	Thr	Phe	Phe	Gly	Ala	Leu	Ala	Ala	Asn	Tyr	Ser	Trp	Leu	Lys
		340						345				350			
Lys	Met	Ser	Ile	Asn	Pro	Glu	Thr	Lys	Ala	Ile	Glu	Gln	Leu	Thr	Val
		355				360						365			

```
<210> SEQ ID NO 11
<211> LENGTH: 458
<212> TYPE: PRT
<213> ORGANISM: Helianthus annuus
```

<400> SEQUENCE: 11

Met Val Ser Pro Ser Ile Glu Val Leu Asn Ser Ile Ala Asp Gly Lys
1 5 10 15

Lys Tyr Ile Thr Ser Lys Glu Leu Lys Lys His Asn Asn Pro Asn Asp
20 25 30

Leu	Trp	Ile	Ser	Ile	Leu	Gly	Lys	Val	Tyr	Asn	Val	Thr	Glu	Trp	Ala	
		35					40					45				
Lys	Glu	His	Pro	Gly	Gly	Asp	Ala	Pro	Leu	Ile	Asn	Leu	Ala	Gly	Gln	
	50					55					60					
Asp	Val	Thr	Asp	Ala	Phe	Ile	Ala	Phe	His	Pro	Gly	Thr	Ala	Trp	Lys	
	65				70				75						80	
His	Leu	Asp	Lys	Leu	Phe	Thr	Gly	Tyr	His	Leu	Lys	Asp	Tyr	Gln	Val	
				85					90					95		
Ser	Asp	Ile	Ser	Arg	Asp	Tyr	Arg	Lys	Leu	Ala	Ser	Glu	Phe	Ala	Lys	
			100					105					110			
Ala	Gly	Met	Phe	Glu	Lys	Lys	Gly	His	Gly	Val	Ile	Tyr	Ser	Leu	Cys	
		115					120					125				
Phe	Val	Ser	Leu	Leu	Leu	Ser	Ala	Cys	Val	Tyr	Gly	Val	Leu	Tyr	Ser	
	130					135					140					
Gly	Ser	Phe	Trp	Ile	His	Met	Leu	Ser	Gly	Ala	Ile	Leu	Gly	Leu	Ala	
				150						155					160	
Trp	Met	Gln	Ile	Ala	Tyr	Leu	Gly	His	Asp	Ala	Gly	His	Tyr	Gln	Met	
				165					170					175		
Met	Ala	Thr	Arg	Gly	Trp	Asn	Lys	Phe	Ala	Gly	Ile	Phe	Ile	Gly	Asn	
			180					185				190				
Cys	Ile	Thr	Gly	Ile	Ser	Ile	Ala	Trp	Trp	Lys	Trp	Thr	His	Asn	Ala	
		195					200					205				
His	His	Ile	Ala	Cys	Asn	Ser	Leu	Asp	Tyr	Asp	Pro	Asp	Leu	Gln	His	
	210				215					220						
Leu	Pro	Met	Leu	Ala	Val	Ser	Ser	Lys	Leu	Phe	Asn	Ser	Ile	Thr	Ser	
					230					235					240	
Val	Phe	Tyr	Gly	Arg	Gln	Leu	Thr	Phe	Asp	Pro	Leu	Ala	Arg	Phe	Phe	
				245					250					255		
Val	Ser	Tyr	Gln	His	Tyr	Leu	Tyr	Tyr	Pro	Ile	Met	Cys	Val	Ala	Arg	
			260					265					270			
Val	Asn	Leu	Tyr	Leu	Gln	Thr	Ile	Leu	Leu	Leu	Ile	Ser	Lys	Arg	Lys	
							280					285				
Ile	Pro	Asp	Arg	Gly	Leu	Asn	Ile	Leu	Gly	Thr	Leu	Ile	Phe	Trp	Thr	
						295					300					
Trp	Phe	Pro	Leu	Leu	Val	Ser	Arg	Leu	Pro	Asn	Trp	Pro	Glu	Arg	Val	
					310					315					320	
Ala	Phe	Val	Leu	Val	Ser	Phe	Cys	Val	Thr	Gly	Ile	Gln	His	Ile	Gln	
				325					330					335		
Phe	Thr	Leu	Asn	His	Phe	Ser	Gly	Asp	Val	Tyr	Val	Gly	Pro	Pro	Lys	
			340					345					350			
Gly	Asp	Asn	Trp	Phe	Glu	Lys	Gln	Thr	Arg	Gly	Thr	Ile	Asp	Ile	Ala	
		355					360					365				
Cys	Ser	Ser	Trp	Met	Asp	Trp	Phe	Phe								

-continued

 Thr Ala Ala Leu Gln Ala Arg Asp Leu Thr Asn Pro Ala Pro Gln Asn
 435 440 445

 Leu Ala Trp Glu Ala Phe Asn Thr His Gly
 450 455

<210> SEQ ID NO 12

<211> LENGTH: 359

<212> TYPE: PRT

<213> ORGANISM: Synechocystis sp.

<400> SEQUENCE: 12

 Met Leu Thr Ala Glu Arg Ile Lys Phe Thr Gln Lys Arg Gly Phe Arg
 1 5 10 15

 Arg Val Leu Asn Gln Arg Val Asp Ala Tyr Phe Ala Glu His Gly Leu
 20 25 30

 Thr Gln Arg Asp Asn Pro Ser Met Tyr Leu Lys Thr Leu Ile Ile Val
 35 40 45

 Leu Trp Leu Phe Ser Ala Trp Ala Phe Val Leu Phe Ala Pro Val Ile
 50 55 60

 Phe Pro Val Arg Leu Leu Gly Cys Met Val Leu Ala Ile Ala Leu Ala
 65 70 75 80

 Ala Phe Ser Phe Asn Val Gly His Asp Ala Asn His Asn Ala Tyr Ser
 85 90 95

 Ser Asn Pro His Ile Asn Arg Val Leu Gly Met Thr Tyr Asp Phe Val
 100 105 110

 Gly Leu Ser Ser Phe Leu Trp Arg Tyr Arg His Asn Tyr Leu His His
 115 120 125

 Thr Tyr Thr Asn Ile Leu Gly His Asp Val Glu Ile His Gly Asp Gly
 130 135 140

 Ala Val Arg Met Ser Pro Glu Gln Glu His Val Gly Ile Tyr Arg Phe
 145 150 155 160

 Gln Gln Phe Tyr Ile Trp Gly Leu Tyr Leu Phe Ile Pro Phe Tyr Trp
 165 170 175

 Phe Leu Tyr Asp Val Tyr Leu Val Leu Asn Lys Gly Lys Tyr His Asp
 180 185 190

 His Lys Ile Pro Pro Phe Gln Pro Leu Glu Leu Ala Ser Leu Leu Gly
 195 200 205

 Ile Lys Leu Leu Trp Leu Gly Tyr Val Phe Gly Leu Pro Leu Ala Leu
 210 215 220

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

 Thr Tyr Gly Ile Val Val Cys Thr Ile Phe Met Leu Ala His Val Leu
 245 250 255

 Glu Ser Thr Glu Phe Leu Thr Pro Asp Gly Glu Ser Gly Ala Ile Asp
 260 265 270

 Asp Glu Trp Ala Ile Cys Gln Ile Arg Thr Thr Ala Asn Phe Ala Thr
 275 280 285

 Asn Asn Pro Phe Trp Asn Trp Phe Cys Gly Gly Leu Asn His Gln Val
 290 295 300

 Thr His His Leu Phe Pro Asn Ile Cys His Ile His Tyr Pro Gln Leu
 305 310 315 320

 Glu Asn Ile Ile Lys Asp Val Cys Gln Glu Phe Gly Val Glu Tyr Lys
 325 330 335

-continued

Val Tyr Pro Thr Phe Lys Ala Ala Ile Ala Ser Asn Tyr Arg Trp Leu
340 345 350

Glu Ala Met Gly Lys Ala Ser
355

<210> SEQ ID NO 13

<211> LENGTH: 448

<212> TYPE: PRT

<213> ORGANISM: *Borago officinalis*

<400> SEQUENCE: 13

Met Ala Ala Gln Ile Lys Lys Tyr Ile Thr Ser Asp Glu Leu Lys Asn
1 5 10 15

His Asp Lys Pro Gly Asp Leu Trp Ile Ser Ile Gln Gly Lys Ala Tyr
20 25 30

Asp Val Ser Asp Trp Val Lys Asp His Pro Gly Gly Ser Phe Pro Leu
35 40 45

Lys Ser Leu Ala Gly Gln Glu Val Thr Asp Ala Phe Val Ala Phe His
50 55 60

Pro Ala Ser Thr Trp Lys Asn Leu Asp Lys Phe Phe Thr Gly Tyr Tyr
65 70 75 80

Leu Lys Asp Tyr Ser Val Ser Glu Val Ser Lys Asp Tyr Arg Lys Leu
85 90 95

Val Phe Glu Phe Ser Lys Met Gly Leu Tyr Asp Lys Lys Gly His Ile
100 105 110

Met Phe Ala Thr Leu Cys Phe Ile Ala Met Leu Phe Ala Met Ser Val
115 120 125

Tyr Gly Val Leu Phe Cys Glu Gly Val Leu Val His Leu Phe Ser Gly
130 135 140

Cys Leu Met Gly Phe Leu Trp Ile Gln Ser Gly Trp Ile Gly His Asp
145 150 155 160

Ala Gly His Tyr Met Val Val Ser Asp Ser Arg Leu Asn Lys Phe Met
165 170 175

Gly Ile Phe Ala Ala Asn Cys Leu Ser Gly Ile Ser Ile Gly Trp Trp
180 185 190

Lys Trp Asn His Asn Ala His His Ile Ala Cys Asn Ser Leu Glu Tyr
195 200 205

Asp Pro Asp Leu Gln Tyr Ile Pro Phe Leu Val Val Ser Ser Lys Phe
210 215 220

Phe Gly Ser Leu Thr Ser His Phe Tyr Glu Lys Arg Leu Thr Phe Asp
225 230 235 240

Ser Leu Ser Arg Phe Phe Val Ser Tyr Gln His Trp Thr Phe Tyr Pro
245 250 255

Ile Met Cys Ala Ala Arg Leu Asn Met Tyr Val Gln Ser Leu Ile Met
260 265 270

Leu Leu Thr Lys Arg Asn Val Ser Tyr Arg Ala His Glu Leu Leu Gly
275 280 285

Cys Leu Val Phe Ser Ile Trp Tyr Pro Leu Leu Val Ser Cys Leu Pro
290 295 300

Asn Trp Gly Glu Arg Ile Met Phe Val Ile Ala Ser Leu Ser Val Thr
305 310 315 320

Gly Met Gln Gln Val Gln Phe Ser Leu Asn His Phe Ser Ser Ser Val

-continued

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

```
<210> SEQ ID NO 14
<211> LENGTH: 446
<212> TYPE: PRT
<213> ORGANISM: Morierella alpina
```

<400> SEQUENCE: 14

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

-continued

Phe	Val	Asn	His	Ile	Asn	Gln	Asp	Met	Phe	Val	Pro	Phe	Leu	Tyr	Gly
				245					250					255	
Leu	Leu	Ala	Phe	Lys	Val	Arg	Ile	Gln	Asp	Ile	Asn	Ile	Leu	Tyr	Phe
			260					265					270		
Val	Lys	Thr	Asn	Asp	Ala	Ile	Arg	Val	Asn	Pro	Ile	Ser	Thr	Trp	His
		275					280					285			
Thr	Val	Met	Phe	Trp	Gly	Gly	Lys	Ala	Phe	Phe	Val	Trp	Tyr	Arg	Leu
	290					295					300				
Ile	Val	Pro	Leu	Gln	Tyr	Leu	Pro	Leu	Gly	Lys	Val	Leu	Leu	Leu	Phe
305				310					315						320
Thr	Val	Ala	Asp	Met	Val	Ser	Ser	Tyr	Trp	Leu	Ala	Leu	Thr	Phe	Gln
			325						330					335	
Ala	Asn	His	Val	Val	Glu	Glu	Val	Gln	Trp	Pro	Leu	Pro	Asp	Glu	Asn
			340					345					350		
Gly	Ile	Ile	Gln	Lys	Asp	Trp	Ala	Ala	Met	Gln	Val	Glu	Thr	Thr	Gln
	355					360						365			
Asp	Tyr	Ala	His	Asp	Ser	His	Leu	Trp	Thr	Ser	Ile	Thr	Gly	Ser	Leu
	370					375					380				
Asn	Tyr	Gln	Ala	Val	His	His	Leu	Phe	Pro	Asn	Val	Ser	Gln	His	His
385					390					395					400
Tyr	Pro	Asp	Ile	Leu	Ala	Ile	Ile	Lys	Asn	Thr	Cys	Ser	Glu	Tyr	Lys
			405						410					415	
Val	Pro	Tyr	Leu	Val	Lys	Asp	Thr	Phe	Trp	Gln	Ala	Phe	Ala	Ser	His
			420					425					430		
Leu	Glu	His	Leu	Arg	Val	Leu	Gly	Leu	Arg	Pro	Lys	Glu	Glu		
		435					440					445			

<210> SEQ ID NO 15
 <211> LENGTH: 4
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: cytochrome b5 domain motif

<400> SEQUENCE: 15

His Pro Gly Gly
1

<210> SEQ ID NO 16
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 16

atggtattac gagagcaaga

20

<210> SEQ ID NO 17
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 17

tctgggatct ctggttcttg

20

-continued

<210> SEQ ID NO 18
 <211> LENGTH: 34
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 18

gcgaagctta aaatggtatt acgagagcaa gagc

34

<210> SEQ ID NO 19
 <211> LENGTH: 33
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: PCR primer

<400> SEQUENCE: 19

gcgggatcca atctaggcaa tcttttttagt caa

33

<210> SEQ ID NO 20
 <211> LENGTH: 443
 <212> TYPE: PRT
 <213> ORGANISM: C. elegans

<400> SEQUENCE: 20

Met Val Val Asp Lys Asn Ala Ser Gly Leu Arg Met Lys Val Asp Gly
 1 5 10 15

Lys Trp Leu Tyr Leu Ser Glu Glu Leu Val Lys Lys His Pro Gly Gly
 20 25 30

Ala Val Ile Glu Gln Tyr Lys Asn Ser Asp Ala Thr His Ile Phe His
 35 40 45

Ala Phe His Glu Gly Ser Ser Gln Ala Tyr Lys Gln Leu Asp Leu Leu
 50 55 60

Lys Lys His Gly Glu His Asp Glu Phe Leu Glu Lys Gln Leu Glu Lys
 65 70 75 80

Arg Leu Asp Lys Val Asp Ile Asn Val Ser Ala Tyr Asp Val Ser Val
 85 90 95

Ala Gln Glu Lys Lys Met Val Glu Ser Phe Glu Lys Leu Arg Gln Lys
 100 105 110

Leu His Asp Asp Gly Leu Met Lys Ala Asn Glu Thr Tyr Phe Leu Phe
 115 120 125

Lys Ala Ile Ser Thr Leu Ser Ile Met Ala Phe Ala Phe Tyr Leu Gln
 130 135 140

Tyr Leu Gly Trp Tyr Ile Thr Ser Ala Cys Leu Leu Ala Leu Ala Trp
 145 150 155 160

Gln Gln Phe Gly Trp Leu Thr His Glu Phe Cys His Gln Gln Pro Phe
 165 170 175

Lys Asn Arg Pro Leu Asn Asp Thr Ile Ser Leu Phe Phe Gly Asn Phe
 180 185 190

Leu Gln Gly Phe Ser Arg Asp Trp Trp Lys Asp Lys His Asn Thr His
 195 200 205

His Ala Ala Thr Asn Val Ile Asp His Asp Gly Asp Ile Asp Leu Ala
 210 215 220

Pro Leu Phe Ala Phe Ile Pro Gly Asp Leu Cys Lys Tyr Lys Ala Ser
 225 230 235 240

-continued

```

Phe Glu Lys Ala Ile Leu Lys Ile Val Pro Tyr Gln His Leu Tyr Phe
      245                      250                      255

Thr Ala Met Leu Pro Met Leu Arg Phe Ser Trp Thr Gly Gln Ser Val
      260                      265                      270

Gln Trp Val Phe Lys Glu Asn Gln Met Glu Tyr Lys Val Tyr Gln Arg
      275                      280                      285

Asn Ala Phe Trp Glu Gln Ala Thr Ile Val Gly His Trp Ala Trp Val
      290                      295                      300

Phe Tyr Gln Leu Phe Leu Leu Pro Thr Trp Pro Leu Arg Val Ala Tyr
305                      310                      315                      320

Phe Ile Ile Ser Gln Met Gly Gly Gly Leu Leu Ile Ala His Val Val
      325                      330                      335

Thr Phe Asn His Asn Ser Val Asp Lys Tyr Pro Ala Asn Ser Arg Ile
      340                      345                      350

Leu Asn Asn Phe Ala Ala Leu Gln Ile Leu Thr Thr Arg Asn Met Thr
      355                      360                      365

Pro Ser Pro Phe Ile Asp Trp Leu Trp Gly Gly Leu Asn Tyr Gln Ile
      370                      375                      380

Glu His His Leu Phe Pro Thr Met Pro Arg Cys Asn Leu Asn Ala Cys
385                      390                      395                      400

Val Lys Tyr Val Lys Glu Trp Cys Lys Glu Asn Asn Leu Pro Tyr Leu
      405                      410                      415

Val Asp Asp Tyr Phe Asp Gly Tyr Ala Met Asn Leu Gln Gln Leu Lys
      420                      425                      430

Asn Met Ala Glu His Ile Gln Ala Lys Ala Ala
      435                      440

```

```

<210> SEQ ID NO 21
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(5)
<223> OTHER INFORMATION: Xaa = any amino acid
<220> FEATURE:
<223> OTHER INFORMATION: histidine box motif

```

```

<400> SEQUENCE: 21

```

```

Gln Xaa Xaa His His
 1              5

```

```

<210> SEQ ID NO 22
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: cytochrome b5-like heme-binding domain motif

```

```

<400> SEQUENCE: 22

```

```

Glu His Pro Gly Gly
 1              5

```

```

<210> SEQ ID NO 23
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

```

-continued

```

<221> NAME/KEY: misc_feature
<222> LOCATION: (1)...(5)
<223> OTHER INFORMATION: Xaa = any amino acid
<220> FEATURE:
<223> OTHER INFORMATION: histidine box motif

<400> SEQUENCE: 23

His Xaa Xaa His His
1           5

```

1. An isolated animal $\Delta 5$ -fatty acid desaturase and functional portions thereof.

2. Isolated *C. elegans* $\Delta 5$ -fatty acid desaturase.

3. A DNA sequence encoding a $\Delta 5$ -fatty acid desaturase according to claim 1 or claim 2.

4. A DNA sequence according to claim 3 and comprising at least a portion of the sequence shown in SEQ.2 and equivalents to that sequence, or to portions of that sequence, which encode a functional $\Delta 5$ -fatty acid desaturase by virtue of the degeneracy of the genetic code.

5. A DNA sequence according to claim 4 derived from a *Caenorhabditis elegans* DNA sequence.

6. A DNA sequence according to claim 3 encoding a functional $\Delta 5$ -fatty acid desaturase and comprising at least a portion of the sequence shown in SEQ.1 and equivalents to that sequence, or to portions of that sequence, which encode a functional $\Delta 5$ -fatty acid desaturase by virtue of the degeneracy of the genetic code.

7. A DNA sequence according to claim 6 derived from a *Mortierella alpina* DNA sequence.

8. A DNA sequence according to any one of claims 3 to 7 wherein the DNA sequence is functional in a mammal.

9. A DNA sequence according to claim 8 in which the DNA sequence is expressed in a mammal

10. A DNA sequence according to claim 9 wherein the DNA sequence is expressed in a human.

11. A DNA sequence obtained by modification of a functional natural gene encoding a $\Delta 5$ fatty acid desaturase according to claim 1 or claim 2.

12. A DNA sequence according to claim 11 wherein the modification includes modification by chemical, physical, or biological means without removing a catalytic activity of the enzyme which it encodes.

13. A DNA sequence according to claim 12 wherein the modification improves a catalytic activity of the enzyme which it encodes.

14. A DNA sequence according to claim 12 or 13 wherein the biological modification includes recombinant DNA methods and forced evolution techniques.

15. A DNA sequence according to claim 14 wherein the forced evolution technique is DNA shuffling.

16. A polypeptide encoded by a DNA sequence according to any of claims 3 to 15.

17. A polypeptide according to claim 16 wherein at least a portion of the polypeptide has the sequence shown in SEQ.3 or functional equivalents to that sequence or to portions of that sequence.

18. A polypeptide according to claim 16 wherein at least a portion of the polypeptide has the sequence shown in SEQ.4 or functional equivalents to that sequence or to portions of that sequence.

19. A polypeptide according to any of claims 16 to 18 wherein the polypeptide catalyses the conversion of dihomogamma linolenic acid to arachidonic acid.

20. A polypeptide according to any of claims 16 to 19 wherein the polypeptide has been modified without removing the catalytic activity of the encoded polypeptide.

21. A polypeptide according to claim 20 wherein the polypeptide has been modified in such a way as to introduce a specific level of saturation of a substrate at a specific location within the molecular structure of the substrate.

22. A vector containing a DNA sequence or any portion of a DNA sequence according to any of claims 3 to 15.

23. A method of producing polyunsaturated fatty acids comprising contacting a suitable substrate with a $\Delta 5$ -fatty acid desaturase according to claim 1 or 2 or a polypeptide according to claim 16 to 21.

24. A method of converting dihomogamma linolenic acid to arachidonic acid wherein said conversion is catalysed by a $\Delta 5$ -fatty acid desaturase according to claim 1 or 2 or a polypeptide or modified polypeptide according to any of claims 16 to 21.

25. An organism engineered to produce high levels of a polypeptide according to any of claims 16 to 21.

26. An organism engineered to produce high levels of a product of a reaction catalysed by a $\Delta 5$ -fatty acid desaturase according to claim 1 or 2 or by a polypeptide according to any one of claims 16 to 21.

27. An organism which has been engineered to carry out the method of claim 23 or claim 24.

28. An organism according to either of claims 26 and 27 wherein the organism is a microorganism.

29. An organism according to claim 28 wherein a microorganism is selected from algae, bacteria and fungi.

30. An organism according to claim 29 wherein a fungi includes phycomycetes.

31. An organism according to claim 28 wherein said microorganism is a yeast.

32. An organism according to any of claims 25 to 27 wherein the organism is a plant.

33. An organism according to claim 32 wherein the plant is selected from oil seed plants and tobacco.

34. An organism according to claim 33 wherein the oil seed plants are selected from oil seed rape, sunflower, cereals including maize, tobacco, legumes including peanut and soybean, safflower, oil palm, coconut and other palms, cotton, sesame, mustard, linseed, castor, borage and evening primrose.

35. A seed or other reproductive material derived from an organism according to claim 33 or claim 34.

36. An organism according to any of claims 25 to 27 wherein the organism is a mammal.

37. An isolated multienzyme pathway wherein the pathway includes a $\Delta 5$ desaturase according to claim 1 or **2** or a polypeptide according to any of claims 16 to 21

38. A compound produced by a conversion of a substrate, wherein said conversion is catalysed by a $\Delta 5$ desaturase according to claim 1 or **2** or by a polypeptide according to any of claims 16 to 21.

39. An intermediate compound produced by the reaction catalysed by a $\Delta 5$ desaturase according to claim 1 or **2** or by a polypeptide according to any of claims 16 to 21.

40. A foodstuff or dietary supplement containing a polyunsaturated fatty acid produced by a method according to claim 23 or **24**.

41. A pharmaceutical preparation containing a polyunsaturated fatty acid produced by a method according to claim 23 or **24**.

42. Prostaglandins synthesised by a biosynthetic pathway including a catalytic activity of a $\Delta 5$ desaturase according to claim 1 or **2** or by a polypeptide according to any of claims 16 to 21.

43. A method for modulation of prostaglandin synthesis by the control of the levels of expression of a DNA sequence according to any of claims 3 to 15.

44. A probe comprising all or part of a DNA sequence according to any of claims 3 to 15 or an equivalent RNA sequence.

45. A diagnostic or search probe comprising all or part of a $\Delta 5$ desaturase according to claim 1 or **2** or of a polypeptide according to any of claims 16 to 21.

46. A method of isolating $\Delta 5$ desaturases using a probe according to claim 44 or **45**.

* * * * *