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(54) **DESATURASE**

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(75) Inventors: **Johnathan A. Napier**, Bristol (GB);
Louise Michaelson, Bristol (GB);
Keith Stobart, Bristol (GB)

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Correspondence Address:

BANNER & WITCOFF
1001 G STREET N W
SUITE 1100
WASHINGTON, DC 20001 (US)

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(73) Assignee: **University of Bristol**, Bristol (GB)

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(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 application No. PCT/GB98/03895, filed on Dec. 23, 1998.

This invention relates to cDNA sequences encoding $\Delta 5$ -fatty acid desaturases comprising the sequences shown in SEQ.1 and SEQ.2.

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

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S54259 : -----MTLSIVK : 7
 S54809 : -----MTSTTSKYT : 9
 S68358 : ILGKVYNVTEWAKEHPGGDAPLINLAGQDVTDAFIA : 72
 S35157 : -----MLTAERIK : 8
 PBOR6 : IQGKAYDVSDWVKDHPGGSFPLKSLAGQEVTDAFYA : 62
 FU2 : IRGRVYDVTKFLSRHPGGVDLLLGAARDVTPVFEM : 62

S54259 : SEDS---SSRPSAVPSDLPLEEDIINTLPSG----- : 35
 S54809 : EGKS-IGFRKELNRRVNAYLEAENISP--RD----- : 37
 S68358 : EHPG-TAKKHLDKLFTGYHLEKDYQVSDISRDRKLA : 107
 S35157 : ETQK-RGFRRVLNQRVDAYFAEHGLTQ--RD----- : 36
 PBOR6 : EHPA-STWKNLDKFFTGYLLKDYSEVSKBYRKLV : 97
 FU2 : YHAFGAADAIMKKYYVGTLSNELPVFPEPTVFHKT : 98

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S54259 : --VFVQDRYKA-----WMTFYIINVVMVG : 56
 S54809 : ---NPPMYLK-----TATILAWVVS : 54
 S68358 : SEFAKAGMFEKKGH-----GVIYELCFVSLILS : 135
 S35157 : ---NPSMYLK-----TLEIVLWBFSS : 53
 PBOR6 : FEFSKMGLYDKKGH-----IMFATLCFLAMLFA : 125
 FU2 : IKTRVEGYETDRDIDPKNRPEIWGRYALIFGSLIAS : 134

6 6 g

S54259 : --LGWLGIAIAPWFLLPVW-VFTGTALTGFFV-IG : 88
 S54809 : --AWTEVVFGPDVLMKLLGCIVLGFVSAVGENES : 88
 S68358 : --ACVYGVLYSGSFWIHMLSGAILGLAWMQIAY-IG : 168
 S35157 : --AWAEVLFAPVIFPVRLLGCMVLAIALAASFENVG : 87
 PBOR6 : --MSVYGVLFCEGVLVHLFSGCLMGFLWIQSGW-IG : 158
 FU2 : YYAQLFVPEVVERTWLQVVFATIMGFACAQVGLNPL : 170

FIG. 1

	HD	H	n	G	g	s	W	
S54259	:	HDCG	HRSF	SRNV	VNDW	VGHILF	-LPIIYPFHSWRI	: 123
S54809	:	HDGN	HGGYSKYQ	WVNYLS	GLTHD--	AIGVESYLKFK		: 122
S68358	:	HDAG	HYQMMATR	GWNK	FAG	FIGNCITG	ISIAWKKW	: 204
S35157	:	HDAN	NAYSSNP	HINRV	LG	MTYD--	FVGLSSFLWRY	: 121
PBOR6	:	HDAG	HYMVVSD	SRLNK	FMGI	FAANCLSG	ISIGWKKW	: 194
FU2	:	HDAS	HFSVTHNPT	VWKIL	GATHD-	FFNGAS	YLVWMY	: 205

	Hn	HH	N	D											
S54259	:	GHN-	QHHKY	TNR	MELD	NAWQ---PWRKE----	E---	: 148							
S54809	:	RHN	VLHHTY	TN	ILGH	VE	TH-GDEL	VRMS	PSME--	Y	: 155				
S68358	:	TNN-	AHH	IAC	NSLD	YD	PD	LQ-HLE	MLAV	SKLF	NSI	: 238			
S35157	:	RHNY	LHHTY	TN	ILGH	VE	TH-GD	GA	VRMS	PE	QE--	H	: 154		
PBOR6	:	NHN-	AHH	IAC	NSL	EYD	PD	LQ-YIE	FL	VV	SK	FF	GSL	: 228	
FU2	:	QHML	GHPY	TN	IAG	AD	PD	VST	FE	DV	RR	IK	PNQ--	K	: 239

	5	6																																		
S54259	:	----	YONAGKEMQV	TYDL	FR	GRA	WIG	SIL	H	W	A	S	I	H	: 180																					
S54809	:	R-	WYHRY	QHW	E	W	FV	Y	P	E	I	P	Y-	YNS	I	A	D	V	Q	T	M	L	F	K	R	: 189										
S68358	:	TSV	FY	G	R	O	L	T	E	D	P	L	A	R	F	F	V	S	Y	Q	H	L	Y	P	I	M	C	V	A	R	I	N	: 274			
S35157	:	V-	GI	R	F	Q	Q	F	Y	I	W	G	L	Y	L	E	I	P	E-	Y	H	F	L	D	V	Y	L	V	L	N	K	G	: 188			
PBOR6	:	TSH	F	Y	E	K	R	L	T	E	D	S	L	S	R	F	F	V	S	Y	Q	H	W	T	F	P	I	M	C	A	A	R	I	N	: 264	
FU2	:	W	F	V	N	H	I	N	Q	D	M	E	V	P	F	L	X	G	L	L	A	F-	K	V	R	I	Q	D	I	N	L	I	F	V	K	: 274

	P																																			
S54259	:	-ED	WTK	F	E	G	K	Q	R	Q	Q	V	K-	F	S	S	L	L	V	I	G	A	A	A	I	A	F--	P	T	M	: 212					
S54809	:	QY	H	D	H	E	I	P	S	P	T	W	V	D	I	A	T	L	L	A	F	K	A	F	G	V	A	V	F	L	I-	I	P	T	A	: 224
S68358	:	L	L	Q	T	I	L	L	L	I	S	K	R	K	I	P-	D	R	G	L	N	I	L	G	T	L	I	F	W	T	W	F	E	L	L	: 309
S35157	:	KY	H	D	H	K	T	P	P	F	Q	P	L	E	A	S	L	L	G	I	K	L	L	W	L	G	Y	V	F	G-	L	P	L	A	: 223	
PBOR6	:	M	V	Q	S	L	I	M	L	L	E	K	R	N	V	S-	Y	R	A	H	E	L	L	G	C	L	V	F	S	I	W	Y	P	L	L	: 299
FU2	:	T	N	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	P	L	Q	: 309	

	6	f	l	H																																	
S54259	:	I	L	T	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	T	T	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	L	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	: D I P F R E P E -----	Q W H E A E S Q L S G T V H C N Y S R W	:	275	
S54809	: P A E F L D E D --	N L H I D D E W A I A Q V K T T V D F A P N - N P I	:	291	
S68358	: D V Y V G P E K G -----	D N W F E K Q T R G T I D I A - C - S S W	:	372	
S35157	: S T E E L T P D G E S G A I D D E W A I C Q I R T T A N F A T N - N P F	:	292		
PBOR6	: S V Y V G K E K G -----	N N W F E K Q T D G T L D I S - C - P P W	:	362	
FU2	: E V Q W P L P D E -	N G I I Q K D W A A M Q V E T T O B Y A H D - S H L	:	376	

	g	6	q	HH6fp	6	C
S54259	: G E F L C H D E N V H I P H R V T T A I P W Y N L R T P T P V Y R K I G	:	311			
S54809	: I N W Y V G G L N Y Q T V H H L E P H I C H I H Y P K I A P I L A E V C	:	327			
S68358	: M D W F F G G L Q F Q L E H H L E P R L P R C H L R S I S P I C R E L C	:	408			
S35157	: W N W E C G G L N H O V T H H L E P N I C H I H Y P Q L E N I K D V C	:	328			
PBOR6	: M D W F H G G L Q F Q L E H H L E P K M P R C N L R K I S E Y V I E L C	:	398			
FU2	: W T S I T G S L N Y Q A V H H L E P N V S Q H H Y P D I L A I K N T C	:	412			

	Y	f	a	6
S54259	: G E Y L Y P E C D F S W G L M K Q V V D H A I C M M R I T I I S Q S L T	:	347	
S54809	: E E F G V N Y A --	V H O T E F G A L A A N Y S W L K K M S I N P -- E	:	359
S68358	: K K Y N L P Y ---	V S L S E Y D A N V T T I K T L K T A A I Q A R D L	:	441
S35157	: Q E E G V E Y K --	V Y P T E K A A I A S N Y R W L E A M G K A S ---	:	359
PBOR6	: K K H N L E Y ---	N Y A S T S K A N E M T E R T L R N T A L Q A R D I	:	431
FU2	: S E Y K V E Y L --	V K D T E W Q A F A S H L E H L R V L G L R P K E E	:	446

S54259	: T K R V -----	:	351
S54809	: T K A I E Q L T V -----	:	368
S68358	: T N P A P Q N L A W E A F N T H - G	:	458
S35157	: -----	:	-
PBOR6	: T K P L P K N L V W E A L H T H G -	:	448
FU2	: -----	:	-

S54259 Δ^{12} *Spirulina*
 S54809 Δ^6 *Spirulina*
 S68358 Putative Sphingolipid desaturase
 S35157 Δ^6 *Synechocystis*
 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*.

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CE5 1 -----MVLREQEHEPFFKIDGRWCQIDDAVLRSHPGGS-AITTYKMDKATIV
CE6 1 -----MVDKN-ASGLRMDKVDGKWLKYLSEELVKKHPFGA-VIEQYRNSDATHI
MA5 1 MGTDQGKFTTWEELAAHNTKGDLELAIRGRVYDVTKFLSR-HPGGVDITLLGAGREVTFV

CE5 48 FHTTEHT-GSKKAYQWTELEKKECPDQEPSPDIDKDEPIKGIIDVNMGTINLSEKRSQAQIN
CE6 47 FHAHHE-GSSQAYKQLDLEKHHG--EHDZFLKQLEKRLDKVDINVSAYDVSVAQEKKMV
MA5 60 FEMTHAFGNADAIMKKYVGTLSVSNLQVFPET-----T-----V--EHKTEKTRVEGY

CE5 107 KSETDLRMRVRAEGIMDGSPLFYTRKILETFTTLEFAYLQYHTTYLPSAIIKQVANDQIL
CE6 104 ESEKLNQKLEHDDGLMKANEITFLFKATSTLSIMAFAYLQYLGWETTSNCLLALANQCF
MA5 107 FDRDIDFKNRPE-IWGRYALIEGSLIASYYAQLFVPEVVERTWLQVVFATIMFACAGV

CE5 167 GWLI-HEFAHQLEFNRYYNLA SYEVGNELQGFBSGGWKEQHNV-HBAATNVVGRDGN
CE6 164 GWLT-HEFCHQPTKNRPLNETHLSLFFGNELQGFSDRWWRDKHNT-HBAATNVVEDHGGQI
MA5 166 GLNPLHDASHFSVTHNP-TVWKILGATHDFENGASYLVVMYQHMGGHPYTNLQAG--AE--

CE5 225 DEVEFYATVAEHLNNSQ--DSWVMTLFRWQVHVHTFMLEFFLRLSNLQSTLHNSQMPH
CE6 222 DLAPLFAFIPGDLCKYRASFEKAILKIVPYQHLVFTAMEPMLRFSWTTGGSVQVVEKENOM
MA5 222 ---EDVSTFEPDVRRIKPN-QKWFVNHIN-QDNEVPTLYGLLAEKVRIGDINILKFKIN

CE5 283 YDYRNTAIDYDVGSLHWANSLGQYFLEDNST----KIMTFLVSHLVGGFLGSRVMT
CE6 282 EYKVYQRNAPWEQATFVGHWANVFTQLFLEPTPL----KVATFELSOMGGGLIYARVMT
MA5 277 DAIRVNPISINHTVMFWGGKATFVWYRLVPLQYLPKGVLELFTVADVSSYWLALTFQ

CE5 339 ENHYSVEREALSIN----TMSNYLAQLQIMTFRNMRP-GRFDNLWGGGLNYQIEHRLTPTM
CE6 338 ENHNSVDKYPANR----ILNNFAALQILTFRNMTF-SPFDNLWGGGLNYQIEHRLTPTM
MA5 337 ANHVVEEVQWPLPDENGLIQKQNAAMQVETFOYAHDSHLWTSITGSLNYQAVHRLTPTV

CE5 394 PRHNENTVMPLVKEFAAANGLPYVDD-YFTGFWLELEQFRNLANVAAKLTKKIA
CE6 393 FPCNLNACVQVKEWCKENNLPLYVDD-YEDGYAMNLQQLKMAEHIQAKA---
MA5 397 SQHHYPDLLALIKNTCSYKVPYLVKETFQAFASHLEHLVGLRPKKE-----
    
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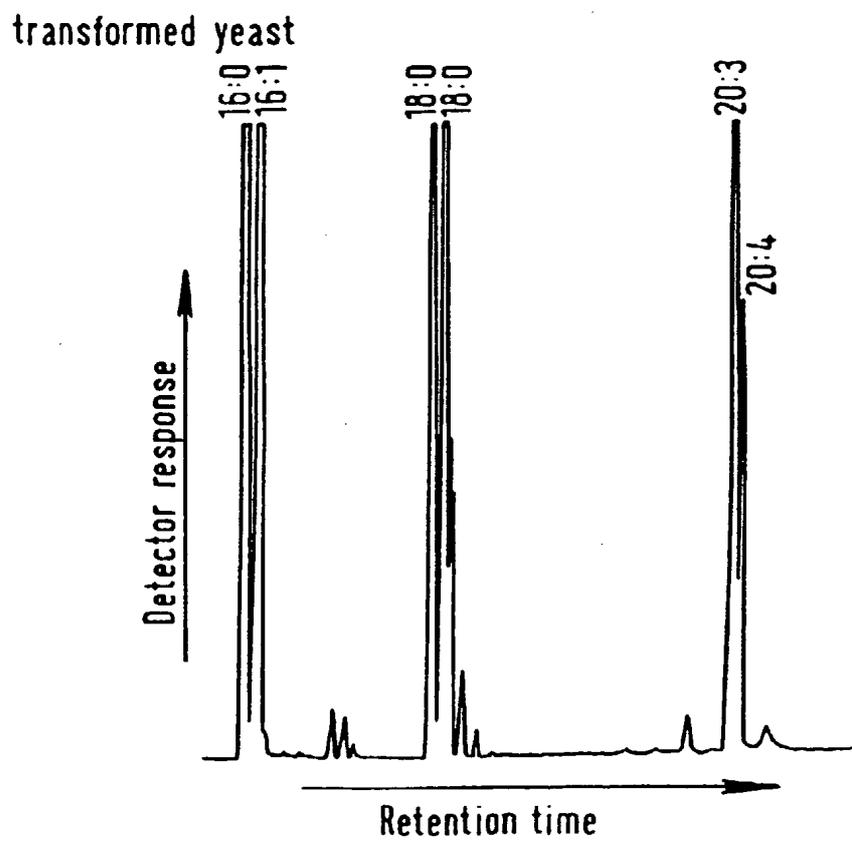
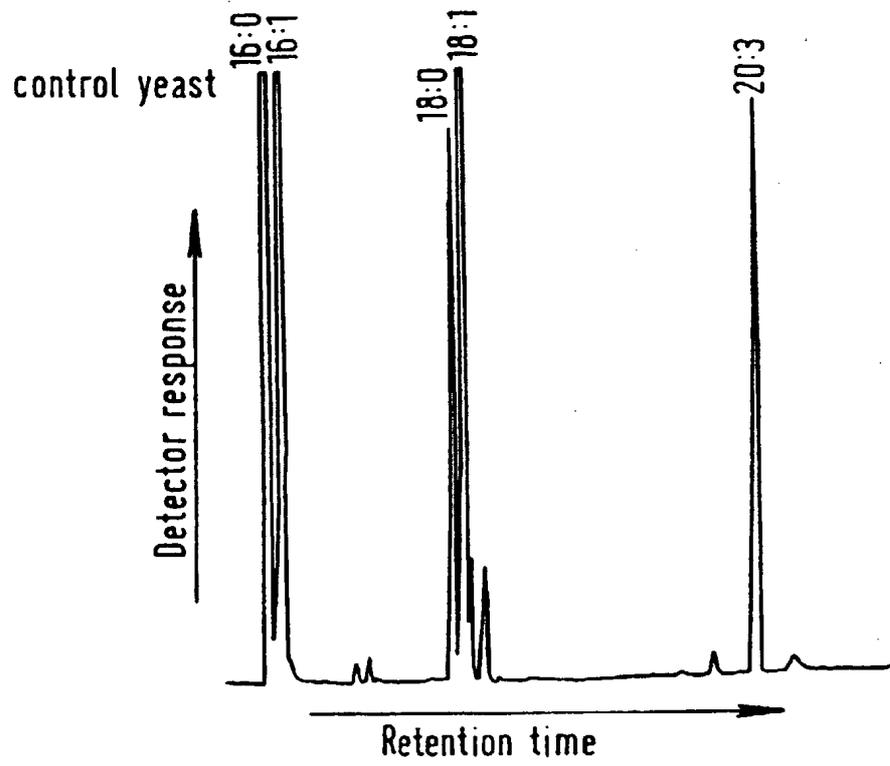


FIG. 3

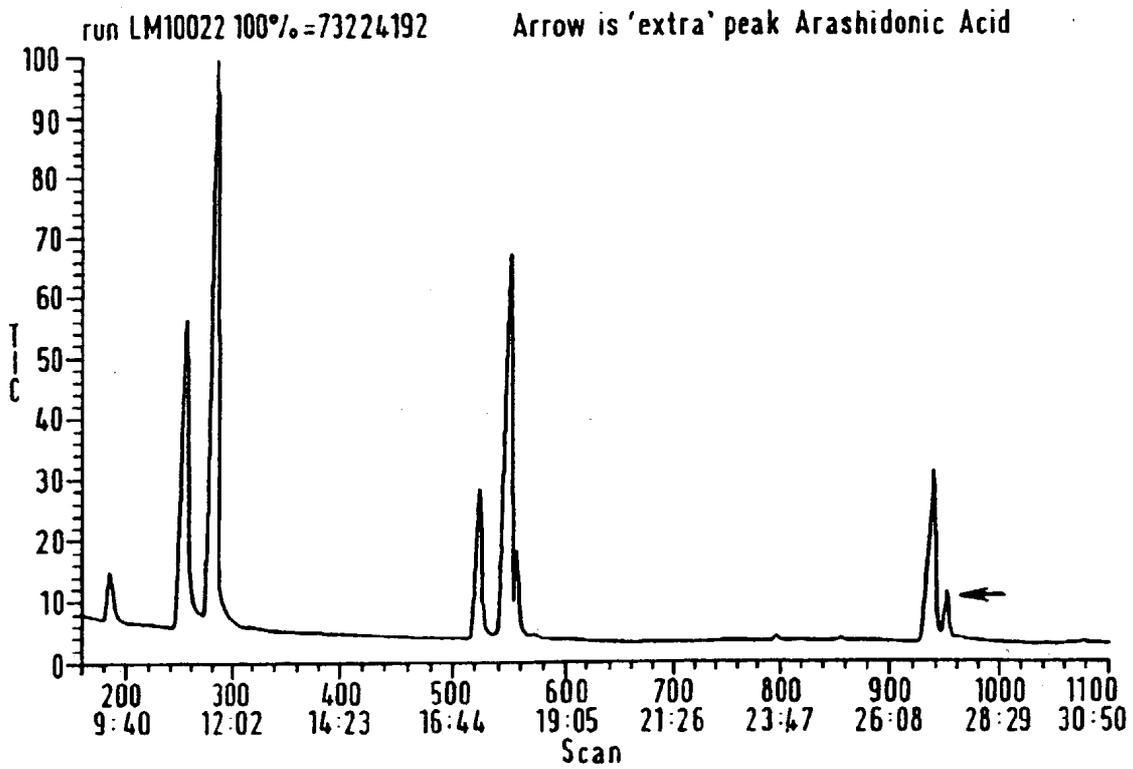
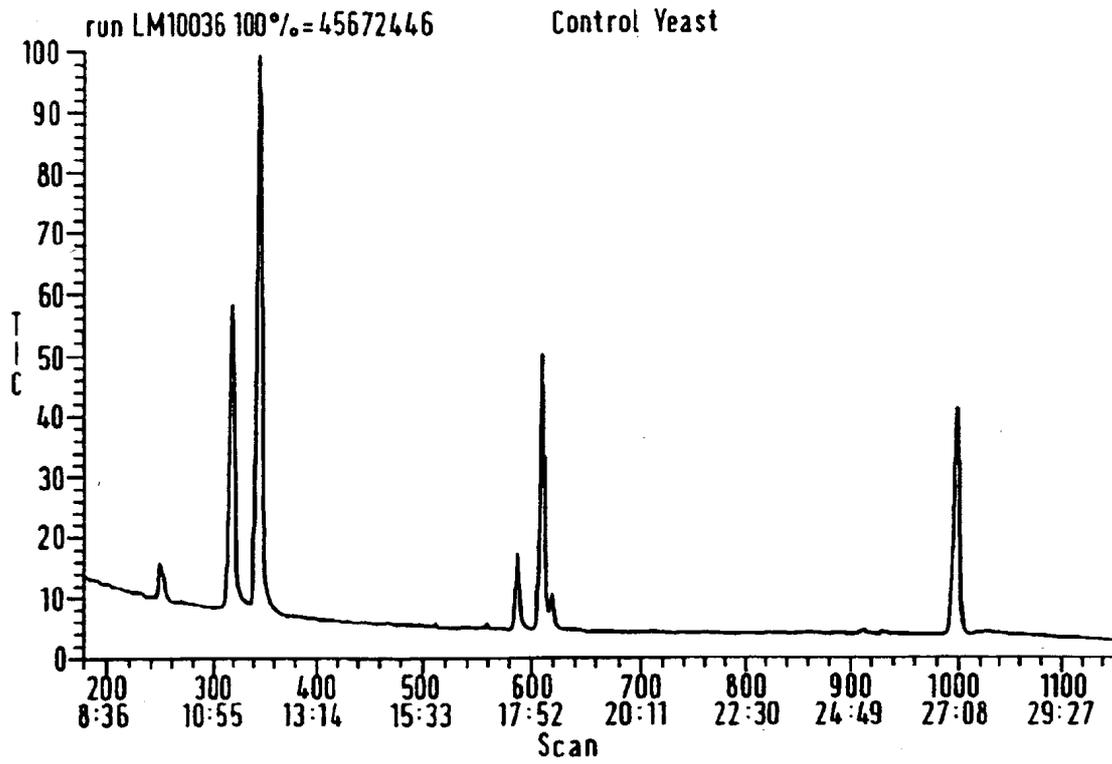


FIG. 4

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 dihomogammna 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), dihomo- γ -linolenate (DHGLA), arachidonic acid (AA), ecosapentaenoate (EPA) and docosahezaenoate (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 phycomyces) 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 eicostatetraenoate (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 $\times 10^7$ 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' - ATGGTATTACGAGAGCAAGA - 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- Labelling 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 Δ ^{9,12}) or di-homo- γ -linolenic acid (C20:3 Δ ^{8,11,14}) in the presence of 1% tertgitol (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 ATTCACACA
 151 TTCCATACTG GTTCTAAAAGA AGCGTATCAA TGGCTGACAG AATTGAAAAA
 201 AGAGTGCCCT ACACAAGAAC CAGAGATCCC AGATATTAAG GATGACCCAA
 251 TCAAAGGAAT TGATGATGTG AACATGGGAA CTTTCAATAT TTCTGAGAAA
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 351 TGCAGAAGGA CTTATGGATG GATCTCCTTT GTTCTACATT AGAAAAATTC
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 501 ATGGTTAATC CATGAATTCG CACATCATCA GTTGTTCAA AACGATACT
 551 ACAATGATTT GGCCAGCTAT TTCGTTGGAA ACTTTTTACA AGGATTCTCA
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 951 TAGAATAATG TTCTTCCTTG TTTCTCATCT TGTTGGAGGT TTCCTGCTCT
 1001 CTCATGTAGT TACTTTCAAT CATTATTGAG TGGAGAAGTT TGCATTGAGC
 1051 TCGAACATCA TGTCAAATTA CGCTTGTCTT CAAATCATGA CCACAAGAAA
 1101 TATGAGACCT GGAAGATTCA TTGACTGGCT TTGGGGAGGT CTTAACTATC
 1151 AGATTGAGCA CCATCTTTTC CCAACGATGC CACGACACAA CTTGAACACT
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 1251 GGTTCGACAT TATTTCACAG GATTCTGGCT TGAATGAG CAATTCGAA
 1301 ATATGCAAAA TGTGCTGCT AAATGACTA AAAAGATTGC CTAG

 1 MGTDQGKTFT WEELAAHNTK GDLFLAIRGR VYDVKFLSR HPGGVDLLLL SEQ. 3
 51 GAGRDVTPVF EMYHAFGAAD AIMKKYVGT LVSNELPVFP EPTVFHKTIK
 101 TRVEGYFTDR DIDPKNRPEI WGRYALIFGS LIASYAQLF VPFVVERTWL
 151 QVVFALIMGF ACAQVGLNPL HDASHFSVTH NPTVWKILGA THDFFNGASY
 201 LVWVYQHMLG HHPYTNIAGA DPDVSTFEPD VVRIKPNQKW FVNHINQDMF
 251 VPFLYGLLAF KVRIQDINIL YFVKTNDAIR VNPISTWHTV MFWGGKAFFV
 301 WYRLIVPLQY LPLGKVLFFF TVADMVSSYW LALTFQANHV VEEVQWPLPD
 351 ENGIQKQDWA AMQVETTQDY AHDShLWTSI TGSLNYQAVH HLFPNVSHH
 401 YPDILAIKKN TCSEYKVPYL VKDTFWQAFV SHLEHLRVLG LRPKEE*

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

1 MVLREQHEP FFIKIDGKWC QIDDAVLRSH PGGSAITTYK NMDATTVFHT SEQ 4

-continued

51 FHTGSKEAYQ WLTELKKECP TQPEEIPDIK DDPIKGIDDV NMGTFNISEK
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 301 HWAWSLGQLY FLPDWSTRIM FFLVSHLVGG FLLSHVVTFN HYSVEKFALS
 351 SNIMSNYAQL QIMTTRNMRP GRFIDWLWGG LNYQIEHHP PTMPRHNLNT
 401 VMPLVKEFAA ANGLEPYMVD YFTGFWEIE QFRNIANVAA KLTKKIA*

[0109]

SEQUENCE LISTING

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cactgggctt ggtcattggg tcaattgat ttctaccg attggtcaac tagaataatg 960
ttctctcttg tttctcatct tgttgagggt ttctgctct ctoatgtagt tactttcaat 1020
cattattcag tggagaagtt tgcattgagc tcgaacatca tgtcaaatta cgcttctct 1080
caaatcatga ccacaagaaa tatgagacct ggaagattca ttgactggct ttggggaggt 1140
cttaactatc agattgagca ccatcttttc ccaacgatgc cagcacaca cttgaacact 1200
gttatgccac ttgtaagga gtttgagca gcaaatggtt taccatacat ggtcgacgat 1260
tatttcacag gattctggct tgaattgag caattccgaa atattgcaa tgttctgtct 1320
aaattgacta aaaagattgc cttag 1344

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

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<400> SEQUENCE: 3

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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

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-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

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<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

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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

gtggTgcaca gctggtagt 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

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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
							70								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
							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
							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
							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
				5					10					15	
Arg	Lys	Glu	Leu	Asn	Arg	Arg	Val	Asn	Ala	Tyr	Leu	Glu	Ala	Glu	Asn
			20					25						30	

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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

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<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

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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
 145 150 155 160
 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
 225 230 235 240
 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
 275 280 285
 Ile Pro Asp Arg Gly Leu Asn Ile Leu Gly Thr Leu Ile Phe Trp Thr
 290 295 300
 Trp Phe Pro Leu Leu Val Ser Arg Leu Pro Asn Trp Pro Glu Arg Val
 305 310 315 320
 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 Gly Gly Leu Gln Phe Gln Leu
 370 375 380
 Glu His His Leu Phe Pro Arg Leu Pro Arg Cys His Leu Arg Ser Ile
 385 390 395 400
 Ser Pro Ile Cys Arg Glu Leu Cys Lys Lys Tyr Asn Leu Pro Tyr Val
 405 410 415
 Ser Leu Ser Phe Tyr Asp Ala Asn Val Thr Thr Leu Lys Thr Leu Arg
 420 425 430

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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

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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

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<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

```

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<400> SEQUENCE: 21

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Gln Xaa Xaa His His
 1             5

```

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<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

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<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.

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