

Supplementary Information

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A non-canonical $\Delta 9$ -desaturase synthesizing palmitoleic acid identified in the thraustochytrid *Aurantiochytrium* sp. T66

Authors

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Supplemental Table S1 Strains used in this study

Strain	Description	Reference
T66	Wild-type <i>Aurantiochytrium</i> sp. T66	ATCC [®]
SR21	Wild-type <i>Aurantiochytrium limacinum</i> SR21	ATCC [®]
#26-1	Transgenic strain derived from SR21 with <i>crtIBY</i> disrupted by <i>ble-2A-T66des9</i> expression cassette	this study
#30-1	Transgenic strain derived from SR21 with <i>crtIBY</i> disrupted by <i>ble</i> expression cassette	this study

Supplemental Table S2 Plasmids used in this study

Plasmids	Description	Reference
pUC19-18GZG	Amp ^R , <i>ble</i> flanked by SR21 endogenous GAPDH promoter and terminator	Addgene
pEMR15	Kan ^R , pCR TM Blunt II-TOPO [®] containing <i>ble</i> (rear part)-2A- <i>T66des9</i> -GAPDH terminator (front part)	This study
pEMR19	Kan ^R , pCR TM Blunt II-TOPO [®] containing <i>crtIBY</i> homologous flanking sequence (upper)	This study
pEMR20	Kan ^R , pCR TM Blunt II-TOPO [®] containing GAPDH promoter- <i>Ble</i> (front part)	This study
pEMR21	Kan ^R , pCR TM Blunt II-TOPO [®] containing GAPDH terminator (rear part)	This study
pEMR22	Kan ^R , pCR TM Blunt II-TOPO [®] containing <i>crtIBY</i> homologous flanking sequence (lower)	This study
pEMR23	Amp ^R , containing GAPDH terminator (rear part)- <i>crtIBY</i> homologous flanking sequence (lower)	This study
pEMR24	Amp ^R , GAPDH promoter- <i>ble-2A-T66des9</i> -GAPDH terminator expression cassette flanked by <i>crtIBY</i> homologous sequences	This study
pEMR26	Amp ^R , GAPDH promoter- <i>ble</i> -GAPDH terminator expression cassette flanked by <i>crtIBY</i> homologous sequences	This study

Supplemental Table S3 Primers used in this study

Primer short name	Primer	Sequence 5'→3'	Underlined region
1	KI2957-1-SmaI	<u>CTCCCGGG</u> ACTTTCGTG	<i>XmaI/SmaI</i>
2	KI2957-2-2a	<u>GTCTCCTGCTTGCTTGAGCAGAGAGAAGTTCGTG</u> <u>GCTCCGGATCCGTCCTGCTCCTCGGC</u>	partial 2A sequence
3	KI2957-3-2a	<u>GCCACGAACTTCTCTCTGCTCAAGCAAGCAGGAG</u> <u>ACGTGGAAGAAAACCCCGGTCCTTGCAAGGTGG</u> AGCCC	partial 2A sequence
4	KI2957-4	TACATGTCGACTCTAGAGGATCCCCTTAGAGCGT CTTGGCCTTG	
5	KI2957-5	GGGGATCCTCTAGAGTCGAC	
6	KI2957-6	CAGAAATA <u>CTAGT</u> TTTGTGAATGAAAAGAGATG ATAAAAG	<i>SpeI</i>
7	GAPDHpro_SbfI-F	AT <u>ACCTGCAGGG</u> GAGACGAGCATATGACTACTG	<i>SbfI</i>
8	Ble_inside_SmaI-R	ACGAAGT <u>CCCGGG</u> GAGAAC	<i>XmaI/SmaI</i>
9	GAPDHter_inside-SpeI-F	CATTCACAAA <u>ACTAGT</u> ATTTCTGCATTAGAAATC	<i>SpeI</i>
10	GAPDHter-NotI-R	ATAG <u>CGGCCG</u> CAGCGGATAACAATTTACACAG G	<i>NotI</i>
11	SR21 CrtUF	<u>GGTACCTGGATGCCTGAGGTCTTC</u>	<i>KpnI</i>
12	SR21 CrtUR	GGGCCCATTAAT <u>CCTGCAGGGA</u> ATACATGGCTGC GCTAC	<i>SbfI</i>
13	SR21 CrtD-NotI-F	ATAG <u>CGGCCG</u> CCGCTCGCTTTCTTGATACTG	<i>NotI</i>
14	SR21 CrtDR	ATTGTCACAGGGCGAACG	
15	21crt-midR	CGGCCGTGTTTCATATAAGAG	
16	21CrtUFcheck	TGGCAGAGCTCATCAGTTTG	
	zeo sjekk-f	AGTTGACCAGTGCCGTTCC	
	zeoRT-r	CGAAGTCGTCCTCCACGAAG	
	SR21tubF2	TGTTGAGAACGCTGATGAGG	
	SR21tubR2	CGAGCTTACGGAGGTCAGAG	
	2957RT-f	GTCAACGCCTACCTCATTGGG	
	2957RT-r	CTTGGAGAAGAGGTGGTGCG	

Supplemental Table S4 T66Des9 homologs that showed the highest degree of identity with proteins encoded by different genera or thraustochytrids species using BLAST in the non-redundant protein sequences database at Genbank

Accession	Description	Species	Query Cover	E value	Per. Ident
AOG21009.1	delta12 desaturase	<i>Thraustochytrium</i> sp. ATCC 26185	100%	0	100.00%
GBG24140.1	Delta12-fatty-acid desaturase	<i>Hondaia fermentalgiana</i>	100%	0	80.65%
BAM37464.1	delta12-fatty acid desaturase (TauΔ12des)	<i>Thraustochytrium aureum</i>	99%	1.00E-124	44.12%
AAO23564.1	delta 12 fatty acid desaturase (PpDes12)	<i>Phaeodactylum tricorutum</i>	97%	8.00E-119	44.34%
BAO27791.1	delta12 desaturase-b	<i>Fistulifera solaris</i>	90%	1.00E-110	44.19%
OEU07512.1	delta 12 fatty acid desaturase	<i>Fragilariopsis cylindrus</i> CCMP1102	91%	2.00E-109	44.25%
XP_002292071.1	predicted protein	<i>Thalassiosira pseudonana</i> CCMP1335	91%	3.00E-106	41.67%
VEU44279.1	unnamed protein product	<i>Pseudo-nitzschia multistriata</i>	90%	2.00E-103	42.57%
CEM11741.1	unnamed protein product	<i>Vitrella brassicaformis</i> CCMP3155	88%	1.00E-101	43.32%
XP_009035621.1	hypothetical protein AURANDRAFT_24467	<i>Aureococcus anophagefferens</i>	87%	2.00E-100	42.01%
MBC8351339.1	fatty acid desaturase	<i>Planctomycetes bacterium</i>	86%	7.00E-93	42.12%
KAA8495721.1	Omega-6 fatty acid desaturase, endoplasmic reticulum isozyme 2	<i>Porphyridium purpureum</i>	96%	8.00E-93	38.69%
NLA37757.1	fatty acid desaturase	<i>Actinobacteria bacterium</i>	85%	7.00E-87	41.32%
XP_002507091.1	fatty acid desaturase	<i>Micromonas commoda</i>	94%	6.00E-85	38.06%
CEO99628.1	hypothetical protein PBRA_007361	<i>Plasmodiophora brassicae</i>	90%	7.00E-84	37.99%
XP_001454547.1	hypothetical protein	<i>Paramecium tetraurelia</i> strain d4-2	85%	2.00E-83	39.28%
NLD75814.1	fatty acid desaturase	<i>Acidimicrobiales bacterium</i>	92%	2.00E-82	39.19%
HBU37186.1	TPA: fatty acid desaturase	<i>Planctomycetaceae bacterium</i>	91%	8.00E-82	37.98%
KAF4668261.1	linoleoyl-CoA desaturase activity	<i>Perkinsus chesapeaki</i>	87%	2.00E-81	38.92%
XP_003075374.1	Fatty acid desaturase, type 1	<i>Ostreococcus tauri</i>	86%	1.00E-80	38.21%
RHZ75025.1	hypothetical protein Glove_218g3	<i>Diversispora epigaea</i>	95%	6.00E-80	34.91%

QDZ17765.1	delta(12)-fatty-acid desaturase	<i>Chloropicon primus</i>	84%	2.00E-78	38.72%
ABQ01458.1	oleate 12-hydroxylase	<i>Physaria lindheimeri</i>	84%	2.00E-78	38.14%
XP_01886021 2.1	delta(12)-fatty-acid desaturase FAD2- like	English walnut	93%	3.00E-78	38.78%
XP_00402505 0.1	hypothetical protein IMG5_193510	<i>Ichthyophthirius multifiliis</i>	91%	6.00E-78	37.28%
KRX08182.1	hypothetical protein PPERSA_12337	<i>Pseudocohnilembus persalinus</i>	88%	7.00E-78	36.19%
GHP07573.1	linoleoyl-CoA desaturase	<i>Pycnococcus provasolii</i>	84%	9.00E-78	40.77%
XP_02264045 9.1	omega-6 fatty acid desaturase, endoplasmic reticulum isozyme 2 isoform X2	mung bean	91%	2.00E-77	39.16%
KAF8643095. 1	hypothetical protein HU200_066967	<i>Digitaria exilis</i>	95%	2.00E-77	38.00%
KAB8088627. 1	hypothetical protein EE612_013286	rice	89%	3.00E-77	38.96%
XP_01722051 8.1	PREDICTED: delta(12)-fatty-acid desaturase FAD2-like	<i>Daucus carota</i> subsp. <i>sativus</i>	90%	4.00E-77	37.86%

Supplemental Table S5 List of thraustochytrid species encoding proteins closely related to T66Des9 by using BLAST in the databases^a at Genbank

Species	nr ^b		wgs ^c	
	Accession	Identity (%)	Accession	Identity (%)
<i>Aurantiochytrium</i> sp. KH105			BGKB01000047.1	87
<i>Aurantiochytrium</i> sp. KH105			BGKB01000033.1	80
<i>Hondaia fermentalgiana</i> strain FCC1311	GBG24140.1	80.65	NPFB01000004.1	81
<i>Schizochytrium</i> sp. CCTCC M209059			JTFK01001013.1	86
<i>Thraustochytrium aureum</i> ATCC 34304	BAM37464.1	44.12	BLSG01000191.1	43
<i>Thraustochytrium</i> sp. ATCC 26185	AOG21009.1	100		

^aNo relevant results were obtained from the sequenced *heterokonts* available at JGI database. ^bThe non-redundant protein sequences database at Genbank. ^cThe *Labyrinthulomycetes* part of the whole genome shotgun database at Genbank

Supplemental Table S6 Fatty acid compositions of *A. limacinum* SR21 strains: all analyzed timepoints ^a

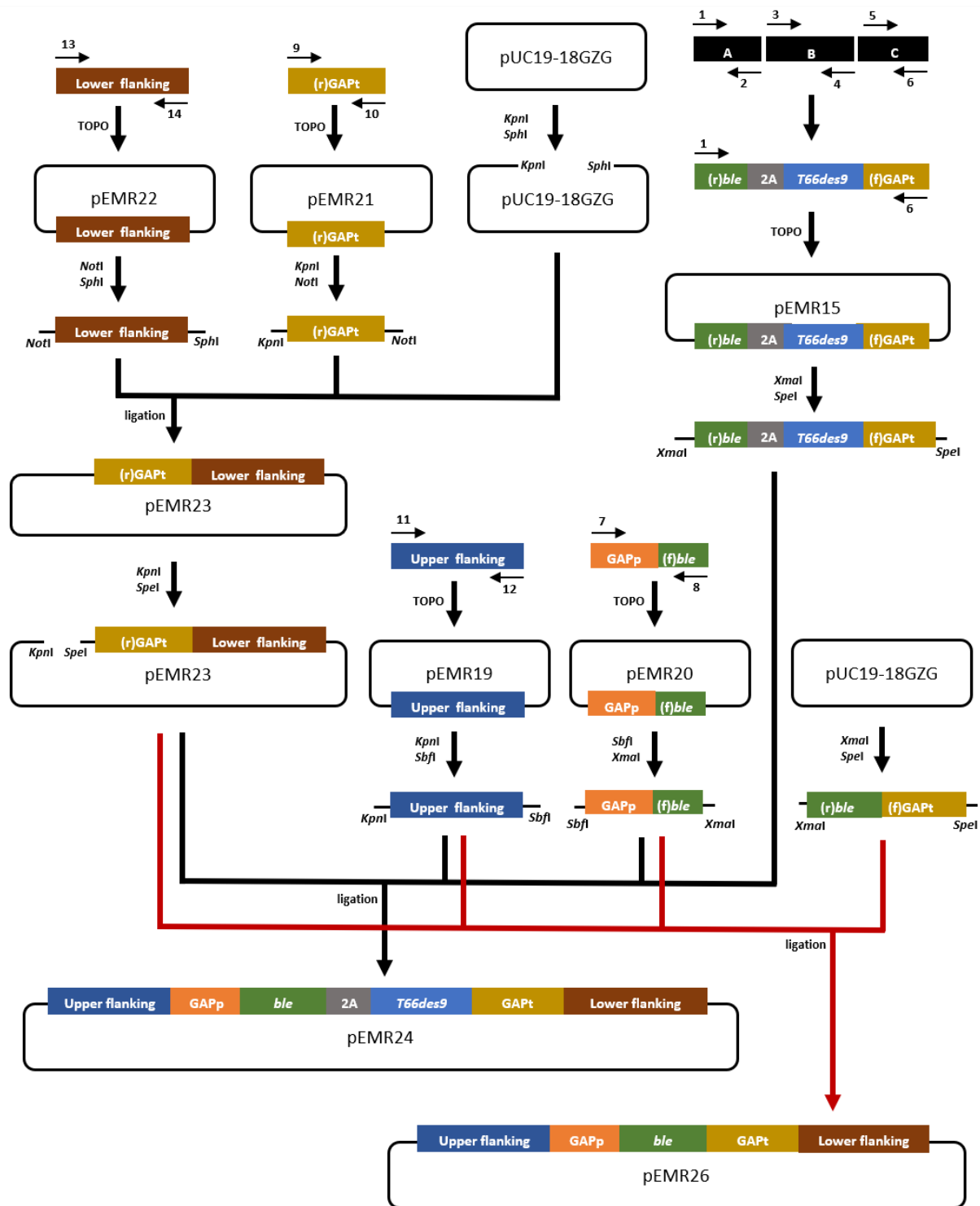
Strain	g/l ^b									
	WT			30-1			26-1			
	Hr FA	16.5	36	60	16.5	36	60	16.5	36	60
C14:0		0.11±0.01	0.28±0.01	0.34±0.01	0.11±0.01	0.31±0.01	0.36±0.01	0.13±0.01	0.34±0.03	0.38±0.04
C15:0		0	0	0	0	0	0	0	0	0
C16:0		1.53±0.06	3.46±0.1	4.71±0.3	1.36±0.12	3.36±0.21	4.35±0.3	1.37±0.23	3.32±0.07	3.99±0.19
C16:1		0	0	0	0	0	0	0.06±0	0.15±0.01	0.2±0.01
C17:0		0	0	0	0	0	0	0	0	0
C18:0		0.02±0.03	0.05±0.07	0.12±0	0.04±0	0.06±0.03	0.09±0.04	0.04±0	0.09±0	0.1±0
C18:1		0	0	0	0	0	0	0.05±0	0.12±0	0.18±0
C20:5		0.02±0	0.02±0	0	0.01±0	0.02±0	0.02±0	0.01±0	0.02±0	0.02±0
C22:5		0.24±0.04	0.58±0.06	0.79±0.08	0.21±0	0.6±0.02	0.77±0.04	0.21±0.01	0.58±0.05	0.71±0.03
C22:6		0.74±0.11	1.8±0.12	2.63±0.48	0.64±0.02	1.78±0.01	2.31±0.18	0.66±0.08	1.66±0.02	2.05±0.14
Total		2.64±0.2	6.18±0.22	8.61±0.72	2.37±0.15	5.32±1.28	6.87±1.85	2.51±0.34	6.21±0.05	7.54±0.35

Strain	% ^c									
	WT			30-1			26-1			
	Hr FA	16.5	36	60	16.5	36	60	16.5	36	60
C14:0		4.18±0.17	4.48±0.26	3.93±0.23	4.51±0.02	5.11±0.22	4.62±0.44	5.01±0.15	5.47±0.45	5.07±0.36
C15:0		0	0	0	0	0	0	0	0	0
C16:0		58.04±2.2 7	55.98±0.3 2	54.72±1.0 2	57.43±1.4 7	54.81±2.1 3	55.18±0.9 2	54.69±1.6 3	53.51±0.6 6	52.94±0.0 2
C16:1		0	0	0	0	0	0	2.36±0.14	2.42±0.13	2.66±0.07
C17:0		0	0	0	0	0	0	0	0	0
C18:0		0.78±1.1	0.74±1.04	1.36±0.06	1.78±0.1	1.03±0.51	1.1±0.54	1.71±0.14	1.44±0.02	1.4±0.09
C18:1		0	0	0	0	0	0	2.08±0.21	1.89±0.07	2.37±0.14
C20:5		0.29±0.01	0.26±0.01	0	0.3±0.01	0.27±0.02	0.3±0.01	0.28±0.01	0.26±0.01	0.29±0.02
C22:5		8.89±0.99	9.37±1.38	9.19±1.65	8.69±0.58	9.75±0.61	9.66±0.74	8.52±0.61	9.31±0.82	9.42±0.86
C22:6		27.82±2.2	29.18±0.9 3	30.47±3.0 7	27.26±0.7 9	29±0.75	29.11±0.8 2	26.57±0.5	26.73±0.4 5	27.13±0.5 7

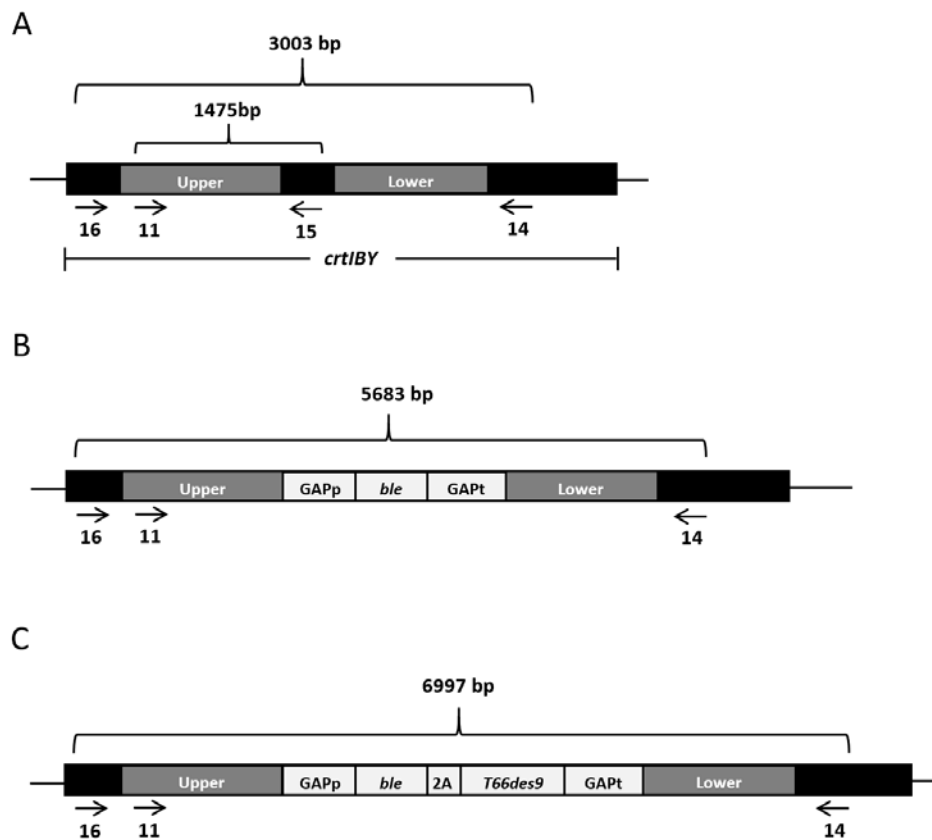
^aData are expressed as the mean ±variation of two replicates originated from two independent cultures. The data of each replicate of strain 30-1 and 26-1 are the mean of two separate runs of FA analysis. ^bGrams per liter of the culture. ^cPercentage of total fatty acids. The FAs less than 0.01 g/l were considered as background signals and were not shown

Supplemental Table S7 Fatty acid compositions of *A. limacinum* SR21 strain 26-1 and *Aurantiochytrium* sp. T66 WT with the isomers distinguished

Fatty acid	Peak Area % of identified FAMES	
	26-1	T66
C14:0	3.76	13.75
C14:1 n-5	< 0.1	< 0.1
C16:0	55.67	32.82
C16:1 n-7	2.64	13.62
C18:0	1.57	1.23
C18:1 n-9	< 0.1	< 0.1
C18:1 n-7	2.45	8.17
C18:3 n-3	< 0.1	< 0.1
C20:0	0.20	< 0.1
C20:4 n-6	0.23	0.15
C20:5 n-3 (EPA)	0.26	0.48
C22:0	0.11	< 0.1
C22:5 n-3 (DPA)	5.99	7.97
C24:0	< 0.1	0.17
C22:6 n-3 (DHA)	26.99	21.42
Sum identified FAMES (%)	99.40	98.19
Sum unknown peaks (%)	0.60	1.65
LOQ 0.1%		



Supplemental Fig. S1 Scheme of constructing pEMR24 and pEMR26. Upper/Lower flanking: *critBY* homologous regions; GAPp: GAPDH promoter; GAPt: GAPDH terminator; (f)/(r)GAPt: the front/rear of GAPt; *ble*: Zeocin resistance gene; (f)/(r)*ble*: the front/rear of *ble*; 2A: peptide self-cleavage sequence; 1~12: PCR primers



Supplemental Fig. S2 Illustration of the sizes of PCR products amplified from the genomic DNA of (A) WT and non-transformants (30-18 and 30-22), (B) strain 30-1, 30-21 and 30-23, (C) strain 26-1. Primer pair 11+15 amplified fragments of 1475bp only from WT, 30-18 and 30-22. Primer pair 16 + 14 amplified larger PCR fragments of 6997bp and 5683bp from 26-1, and the three strains (30-1, 30-21 and 30-23), respectively. Primer pair 16 and 14 amplified a shorter fragment of 3003bp from WT, 30-18 and 30-22. Upper/Lower: *crtIBY* homologous regions; GAPp/t: GAPDH promoter/terminator; *ble*: Zeocin resistance gene; 2A: peptide self-cleavage sequence; Arrows: primer annealing sites