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MOLECULAR SWITCH THAT CONTROLS THE FLUX OF LINOLEIC ACID INTO N-6 OR N-3 POLYUNSATURATED FATTY ACIDS IN MICROORGANISMS

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ABSTRACT

Polyunsaturated Fatty Acids (PUFA) of the n-6 and n-3 series play important roles in nutrition. Microorganisms are important sources of n-6 and n-3 fatty acids; however, most produce either n-6 or n-3 fatty acids as the major PUFAs and very few produce both. This differential production suggests that PUFAs metabolic pathway is strictly controlled in microorganisms. The major pathway of n-6/n-3 faty acids biosynthesis in lower eukaryotes is composed of Δ12 Desaturase (Des), ω3 Des (Δ15, Δ17), Δ6 Des, Δ6 Elongase (Elo), Δ5 Des, Δ5 Elo and Δ4 Des, among which Δ6 Des and Δ15 (ω3) Des, located at the branch point of PUFAs metabolic pathways, are key regulators of the flux of linoleic acid (18:2 n-6) into either n-6 or n-3 fatty acid metabolic pathways. These latter two enzymes work together as a molecular switch that control the production of n-6/n-3 fatty acids. However the mechanism of the molecular switch is, so far, not clear. This review summarizes the recent advancement of the molecular base of the differentail production of n-6 or n-3 PUFAs in microorganisms.

Keywords: Poly Unsaturated Fatty Acids (PUFAs), n-6 PUFAs, n-3 PUFAs, Metabolic Flux, Microorganisms

1. INTRODUCTION

Poly Unsaturated Fatty Acids (PUFAs) are straight chain fatty acids with chain lengths varying from C18 to C22 and more than two double bonds. Among these, n-6 and n-3 fatty acids are essential to human health and play important but different physiological roles. A balanced ratio of n-6/n-3 fatty acids and the amount of n-3 fatty acids, is regarded as important factors for human health (Harris, 2006; Scott *et al*., 2007; Candela *et al*., 2011). However the ratio of n-6/n-3 in current diet oils is commonly around 10:1 to 25:1, which is far from ideal, due to the fact that most of the traditional plant oils, such as soybean oil, sunflower oil, peanut oil, contains high-levels of n-6 fatty acid, linoleic acid (LA; 18:2 n-6)), with the exception of a few plant oils, such as *Linum usitatissimum* and *Perilla frutescens*, that contain very high amounts of n-3 fatty acid, Alpha-Linoleic Acid (ALA; 18:3 n-3). Furthermore, most plant species do not usually produce Δ6-desaturated fatty acids, with a few exceptions such as *Boraginaceae*, *Oenothera* spp. and *Ribes* spp. (Gunstone, 1992). In addition, some members of the *Primulaceae* family accumulate γ-linolenic acid (GLA; 18:3 n-6) and StearidDonic Acid (SDA; 18:4 n-3) (Sayanova *et al*., 1999; 2006). *Primula* species can produce either n-6 or n-3 Δ6-desaturated fatty acids (GLA or SDA) depending on the substrate preference of Δ6 Desaturase (Δ6 Des) but the overall production of n-3 fatty acids is much higher than n-6 fatty acids suggesting a high activity of ω-3 Desaturase (ω-3 Des) is present in these organisms (Sayanova *et al*., 1999; 2003; 2006). However, in general, higher plants does not produce PUFAs with carbon chain length beyond C18 and the few that do, do not produce fatty acids with more than two double bonds.

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Very long chain PUFAs (VL-PUFAs, with carbon chain length ≧20) commonly occur in many microorganisms including microalgae (Lenihan-Geels *et al*., 2013), filamentous fungi (Shimizu *et al*., 1988), marine protists Schizochytrium and Thraustochytrium (traditionally refered to marine fungi) (Nagano *et al*., 2011) and bacteria (Abd Elrazak *et al*., 2013). In microalgae and filamentous fungi, the biosynthetic pathway for PUFAs is the desaturase/elongase system, while in marine protsits, the desaturase/elongase system and Polyketide Synthase (PKS) both exist and in marine bacteria only PKS is available for PUFAs biosynthesis (Metz *et al*., 2001). Therefore in this review we focus on the regulation of PUFAs biosynthesis in microalgae and filamentous fungi. Most oleaginuous microorganisms, such as algae and a few fungi, produce oils with n-3 fatty acids as dominant PUFAs, some, such as the zygomycete fungi, produce oils with n-6 fatty acids as dominant PUFAs, only very few microorganisms can produce oils with both n-3 and n-6 fatty acids as major PUFAs. Therefore the production of different series of PUFAs is strictly regulated in each individual microorganism, suggesting that the metabolic flux of LA to n-6/n-3 fatty acids is controlled by endogeneous biosynthetic pathways which are regulated by either the property of the enzymes, such as their substrate preference, or environmental stimuli such as low temperature, which has been shown to induce n-3 fatty acid prodution (Shimizu *et al*., 1988). The main enzymes involved in the biosynthesis of n-6/n-3 PUFAs in eukaryotic microorganisms (both algae and fungi) are: Δ12 Des, ω-3 (Δ15, Δ17) Des, Δ6 Des, Δ6 Elo, Δ5 Des, Δ5 Elo and Δ4 Des (Pereira *et al*., 2003), as shown in **Fig. 1**.

During the synthesis of PUFAs, the precursor, oleic acid (18:1 n-9) is first converted to LA (18:2 n-6) by Δ12 Des which, in turn, is converted to ALA (18:3 n-3) by Δ15 Des. LA and ALA are further converted to Docosapentaenoic Acid (DPA, 22:5 n-6) and Docosahexaenoic Acid (DHA, 22:6 n-3), respectively, as the end products of the n-6/n-3 fatty acid pathways catalysed by Δ6 Des, Δ6 Elo, Δ5 Des, Δ5 Elo and Δ4 Des. Among these enzymes, Δ6 Des and ω-3 Des (especially Δ15 Des), located at the branch point of n-6/n-3 fatty acid pathways, work together as a molecular switch that regulate the flux of LA into either n-6 or n-3 fatty acid pathway.

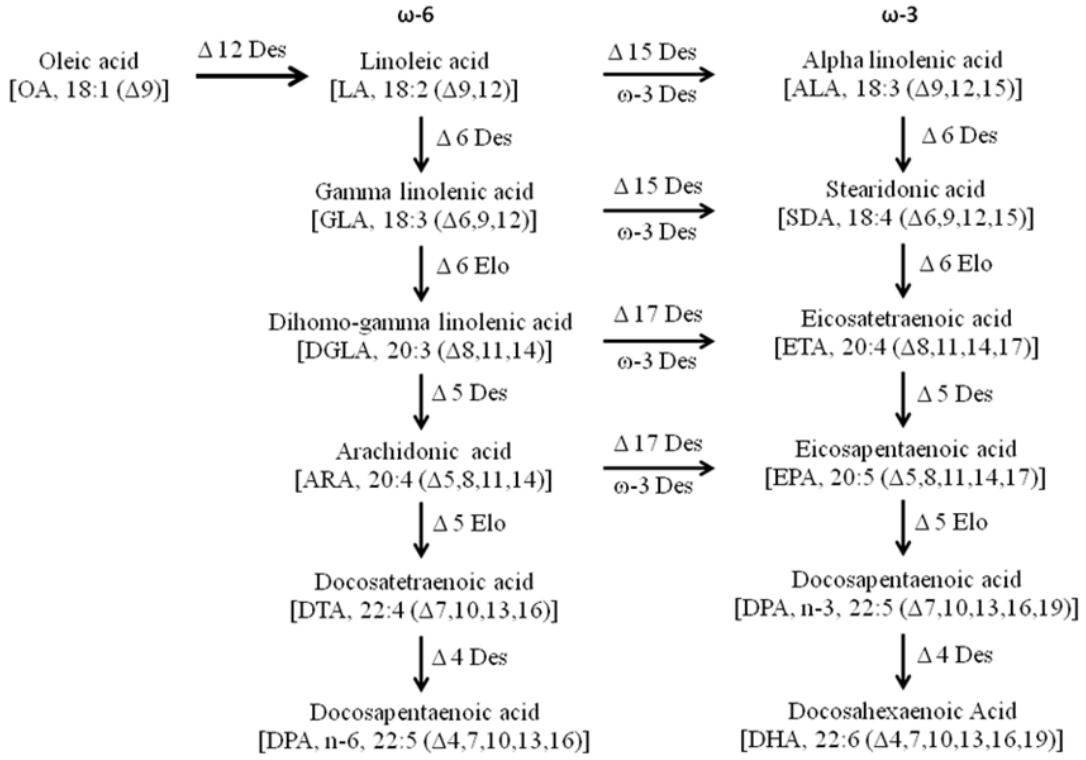

Fig. 1. Main metabolic pathway of LA flux into n-6 or n-3 PUFAs in eukaryotic microorganisms

In this article, we summarize the recent findings about n-6/n-3 fatty acid production in microorganisms that contain both Δ6 Des and ω-3 Des. We also give a summary of the biochemical characterization of Δ6 Des and ω-3 Des and provide a rational correlation between the LA flux and the substrate preference and activity of the molecular switch.

2. MICROORGANISMS THAT CONTAIN BOTH Δ6 DES AND Ω-3 DES GENES

Not all PUFAs-producing microorganisms with desaturase systems contain both Δ6 Des and ω-3 Des genes. For example, the filamentous fungi *Mucor circinelloides* that produces significant amounts of GLA (Kennedy *et al*., 1993), but not ALA or SDA, has the Δ6 Des gene but not that coding for Δ15 (ω-3) Des in its genome as revealed by genomic analysis (http://genome.jgi-psf.org/Mucci2/Mucci2.home.html). In contrast, the green microalga, *Chlamydomonas reinhardtii*, that produces 80% of its total fatty acids as n-3 fatty acid ALA (18:3 n-6) has ω-3 Des gene but not Δ6 Des gene in its genome (Nguyen *et al*., 2013). Neither of these microorganisms has both Δ6 Des and ω-3 Des and clearly no switching between the PUFA pathways is possible. Therefore, these and similar oleaginous microorganisms that only produce PUFAs of a single series will not be further discussed in this paper.

Although genomic sequences of many fungi and microalgae are available, the desaturases of those organisms have yet to be identified. Those organisms that have both Δ6 and ω-3 Des, based on the PUFAs profile of the microorganisms, including some where molecular work has been carried out on their desaturases, are listed in **Table 1**. Most microorganisms that have both Δ6 Des and ω-3 Des are filamentous fungi and microalgae. In general, yeast species have Δ15 (ω-3) Des but not Δ6 Des in their genome; however, one yeast, Candida diddensiae, may have both Δ6 and ω-3 Des based on its fatty acid profile (Rateldge, 1997). This, however, is from a single report and the constituent desaturases were not identified. Interestingly, cyanobacteria species show highly diversified desaturase systems (Dyal and Narine, 2005): Group 1 have only Δ9 Des and, as a consequence, they produce only monounsaturated fatty acids; group 2 have Δ9 Des, Δ12 Des and Δ15 (ω-3) Des and, accordingly, these organisms can produce LA, that is further converted to n-3 fatty acid ALA (18:3 n-3); group 3 contain Δ9 Des, Δ12 Des and Δ6 Des and produce LA, that is further converted to GLA (18:3 n-6); group 4 contain Δ9 Des, Δ12 Des, Δ15 (ω-3) Des and Δ6 Des, therefore the LA produced in this group of cyanobacteria can be converted into both n-6 GLA and n-3 ALA.

3. MOLECULAR SWITCH OF MICROORGANISMS THAT PRODUCE N-6 FATTY ACIDS AS MAJOR LIPIDS

Species belonging to the genus *Mortierella* can produce large amounts of Arachidonic Acid (ARA; 20:4 n-6) but very little Eicosapentaenoic Acid (EPA; 20:5 n-3) (Murata and Wada, 1995). *Mortierella alpina* is currently used to produce ARA commercially. Genomic analysis has shown that this fungus contains Δ15 (ω-3) Des, Δ6 Des, Δ6 Elo and Δ5 Des fatty acid desaturation/elongation systems for the production of series PUFAs (Wang *et al*., 2011). Although it contains a complete set of desaturase genes for the biosynthesis of both ARA (n-6) and EPA (n-3), at physiological growth temperatures, it produces ARA as the only major VL-PUFA. This regulation of PUFA metabolic flux is partly due to the substrate specificity of the Δ6 Des **Table 2**, (Liu *et al*., 2011; Sakuradani and Shimizu, 2003; Sakuradani *et al*., 2005; Zhu *et al*., 2002) which prefers n-6 fatty acid LA to n-3 ALA as substrate and its ω-3 Des must be repressed at physiological growth temperature (Shimizu *et al*., 1998). Although the ω-3 Des have been cloned and biochemically studied in a few microorganisms **Table 5**, (Gellerman and Schlenk, 1979; Pereira *et al*., 2004; Sakamoto *et al*., 1994; Wada and Murata, 1990), its expression level in oleaginous microorganisms has not been determined so far. However over-expression of ω-3 Des in *Mt. alpina* by genetic engineering increased the production of EPA from 5 to 35% of the total FAs and ARA decreased from 60 to 30% of total FAs, while the amount of total fatty acids did not change significantly (Ando *et al*., 2009). These results suggested that the Δ6 Des and ω-3 Des work together as a switch, which is regulated by their substrate preference and activity, controlling the flux of LA into n-6 and/or n-3 fatty acid pathways. Furthermore, at low temperatures (12-14°C) some species of *Mt. alpina* can produce significant amount of EPA (2% w/w of TFA for strain ATCC 32222; 15% w/w of TFA for strain IS-4). These results suggested that ω-3 Des, which converts LA to ALA, is regulated by temperature. Whereas cold-induced expression of ω-3 Des gene at mRNA level have been found in algae and many plants (Wada and Murata 1990; Shi *et al*., 2011; Zhang *et al*.,

2011; Takemura *et al*., 2012) and it is most sensitive to changes in temperature compared to other desaturases such as Δ9, Δ12 and Δ6 Des gene (Sakamoto *et al*., 1994). Therefore, it is suggested that ω-3 Des gene in *Mt. alpina* is repressed at physiological temperature of 25-28°C, leading to flux of LA to ARA and at low temperature, it is induced, leading to the partial flux of LA to ALA, which is further metabolized to EPA.

Recent work has found that a phototrophic alga, *Parietochloris incisa*, can produce ARA up to 60% of the total FA, but with very little EPA **Table 4**, (Iskandarov *et al*., 2010), although the Δ6 Des of this organism can convert LA and ALA at similar activities. This suggests that its Δ15 or ω-3 Des gene must be repressed at physiological temperatures, which resulting in the switch-off of LA flux to ALA and therefore leading to diminished flux of ALA to EPA. Similarly, other organisms such as *Synechocystis* sp. PCC 6803 and *Conidiobolus obscurus* that can produce more n-6 fatty acids than n-3 fatty acids, even though their Δ6 Des works equally with either n-6 or n-3 fatty acid **Table 3**, (Reddy *et al*., 1993; Tan *et al*., 2011).

Pythium irregulare, is a rare example of a microorganism that can produce both n-6 ARA and n-3 EPA as its major PUFAs **Table 4**, (O'Brien *et al*., 1993; Hong *et al*., 2002). The Δ6 Des of this fungus can catalyze the desaturation of both LA and ALA at high conversion yields, albeit with slight substrate preference towards LA. These results indicate that a ω-3 Des is actively expressed in this organism. Furthermore, the fungus produced much more EPA than ARA (1.4-fold increase) when grow at low temperature state, likely due to the induction of ω-3 Des gene by low temperature.

4. MOLECULAR SWITCH OF MICROORGANISMS THAT PRODUCE N-3 FATTY ACID AS MAJOR LIPIDS

Many oleaginous microalgae can produce n-3 VL-PUFAs and the Δ6 Des from several n-3 fatty acid producing organisms have been characterized biochemically **Table 3**, (Dunstan *et al*., 1992; Domergue *et al*., 2005; Petrie *et al*., 2010a; 2010b; Wagner *et al*., 2010; Ahmann *et al*., 2011). In general, the substrate preference of Δ6 Des correlates with the ratio of n-3/n-6 fatty acids. Marine microalgae are one of the primary producers of n-3 fatty acids, such as EPA and DHA and the ratio of n-3/n-6 fatty acids in these organisms are usually very high. In terms of the substrate specificities of the Δ6 Des in these organisms, as shown in **Table 3**, they usually prefer to use n-3 fatty acids ALA. For example, Δ6 Des from *Ostreococcus lucimarinus* showed strong preference for ALA, 39% of fed ALA was converted to SDA, while only 6.6% of fed LA was converted to GLA. Δ6 Des from *Micromona pusilla* also shows strong preference for n-3 fatty acids, the conversion yield of ALA is ten times higher than that of LA.

These data suggests that the substrate preference of Δ6 Des play a major role in directing the flux of LA into n-3/n-6 fatty acid pathway. However, it is noteworthy that Δ6 Des from microalgae *Phaeodactylum ricornutum*, *Glossomastix chrysoplasta* and *Thalassiosira pseudonana* have no significant substrate preference (**Table 4**), but these organisms can still produce n-3 fatty acids as major PUFAs, indicating that an active ω-3 Des gene is highly expressed in these organisms, although the ω-3 Des from these organisms have not been characterized so far. Taken together, the n-3 fatty acid preference of Δ6 Des and the potential high-level expression of ω-3 Des gene probably lead to the high production of n-3 fatty acids and thus high ratio of n-3/n-6 fatty acids.

5. POTENTIAL REGULATORY MECHANISM OF THE MOLECULAR SWITCH

The molecular switch of n-6/n-3 PUFA production is controlled by the substrate preference of Δ6 Des and the activity of ω-3 Des. Although many studies have been carried out to determine the substrate preference of heterogeneous expressed Δ6 Des, no structure-function studies have been achieved so far, not to mention how this substrate preference of Δ6 Des may be regulated by nutritional or environmental conditions.

The molecular mechanism for the sensing of low-temperature and induced expression of desaturase gene in eukaryotic organisms is unknown, however it has been investigated in cyanobacteria and *Bacillus subtilis* (Suzuki and Murata, 2000; Aguilar *et al*., 2001) and well reviewed (Sakamoto and Murata, 2002; Shivaji and Prakash, 2010). It was suggested that the primary signal perceived by a bacterium when exposed to low-temperature is the rigidification of the membrane. This causes the activation of a membrane-associated sensor, which in turn, activates a cytosolic response regulator. Besides, this activated response regulator binds to the promoter of the desaturase and as a consequence the desaturase is induced. In cyanobacteria the Hik 33 (sensor)-Rer 26 (response regulator), while in Bacillus the DesK (sensor)-DesR (response regulator), the two component system for low-temperature signaling has been identified (Suzuki and Murata, 2000; Aguilar *et al*., 2001).

Table 1. Microorganisms that contain both Δ6 Des and ω-3 Des

	Family	Species	Main PUFAs (%)	References
Cyanobacteria	?	*Synechocystis* sp. PCC 6803	GLA: 21 SDA: 8	(Wada and Murata, 1990; Reddy *et al*., 1993; Sakamoto *et al*. 1994)
Yeast	Saccharomycetaceae	*Candida diddensiae*	ALA: 5 SDA: 1 GLA: 10	(Rateldge, 1997) (Shimizu *et al*., 1988; Sakuradani *et al*., 2005)
	*Mortierella*ceae		GLA: 37	
		Mortierella elongate	ARA: 5 EPA: 7 ARA: 11[a]	(Bajpai *et al*., 1992)
			EPA: 14[a]	(O'Brien *et al*., 1993;
		Pythium irregular	ARA: 10[b] EPA: 25[b]	Hong *et al*., 2002)
Filamentous fungus	Pythiaceae	*Pythium ultimum*	ARA: 15 EPA: 12 GLA: 2	(Weete and Gandhi, 1992)
		Phytophthora infestans	ARA: 3 EPA: 20 GLA: 3 DGLA: 7	(Larkin and Groves, 2003; Sun *et al*., 2013)
	Ancylistaceae	*Conidiobolus obscures*	ARA: 10 ETA: 1 EPA: 7	(Tan *et al*., 2011)
	Saprolegniaceae	*Saprolegnia diclina*	ARA: 10 EPA: 20 SDA: 21	(Gellerman and Schlenk, 1979; Pereira *et al*., 2004)
		Micromonas pusilla	EPA: <1 DPA: <1 DHA: 9	(Dunstan *et al*., 1992; Petrie *et al*., 2010b)
	Mamiellaceae	*Ostreococcus lucimarinus*	DHA: 3	(Petrie *et al*., 2010a; Ahmann *et al*., 2011)
Microalgae		*Ostreococcus tauri*	DHA: 12	(Domergue *et al*., 2005;
	Phaeodactylaceae	*Phaeodactylum tricornutum*	ETA: <1 ARA: 1 EPA: 36 DHA: 3	Wagner *et al*., 2010) (Grima *et al*., 1996; Domergue *et al*., 2002)
	Thalassiosiraceae	*Thalassiosira pseudonana*	EPA: 17 DHA: 5	(Tonon *et al*., 2002; Tonon *et al*., 2005)
	Pinguiochrysidaceae	*Glossomastix chrysoplasta*	ARA: 6 EPA: 39 DHA: 9	(Kawachi *et al*., 2002; Hsiao *et al*., 2007)
		Parietochloris incise	ARA: 57 EPA: <1	(Iskandarov *et al*., 2010)
	Chlorellaceae	*Chlorella minutissima*	ARA: 3 EPA: 45	(Seto *et al*., 1984)
		Chlorella NKG042401	GLA: 11 ALA: 14 ARA: 5 EPA: 2	(Hirano *et al*., 1990)
		Schizochytrium sp.	DPA: <1 DHA: 6	(Nagano *et al*., 2011)
	Thraustochytriaceae		ARA: 5 EPA: 3	
		Thraustochytrium sp.	DPA: <1 DHA: 29 ARA: 1	(Nagano *et al*., 2011)
	Isochrysidaceae	*Isochrysis galbana*	EPA: 25 DHA: 11	(Liu *et al*., 2013)
		Nannochloropsis oculata	ARA: 4 EPA: 38	(Tonon *et al*., 2002) (Tonon *et al*., 2002)
	Monodopsidaceae	*Nannochloropsis sp.*	ALA: 2 ARA: 5 EPA: 28	(Nitsan *et al*., 1999)

Table 2. Δ6 Des with substrate preference of LA

Organisms	Main PUFAs (%)	n-3 FAs/n-6 FAs	Δ6 Des substrate conversion yield§ (%) LA	ALA	Reference
Mt. alpina 1S-4	ARA: 48* EPA: 0* GLA: 10† ARA: 28† EPA: 15†	0[a] <1[b]	47	30	(Shimizu *et al*., 1988; Sakuradani and Shimizu, 2003) (Zhu *et al*., 2002; Liu *et al*., 2011)
Mt. alpina W15	GLA: 4 DGLA: 3 AA: 9	0	51	0	

*Strain was cultivated at 28°C†Strain was cultivated at 12°C §Substrate conversion yield was calculated as described previously (Sakuradani *et al*., 2005), Conversion yield (%) = 100× ([product]/ [product + substrate]. Δ6 Desaturase gene from *M. alpina* 1S-4 was expressed in *A. oryzae*. Δ6 Des gene from *M. alpina* W15 was expressed in *P. pastoris*. The substrate preference was calculated based on its endogenous fatty acids production

Table 3. Δ6 Des with substrate preference of ALA

Organisms	Main PUFAs (%)	n-3 FAs/n-6 FAs	Δ6 Des substrate conversion yield§ (%) LA	ALA	Reference
Micromonas pusilla EPA:<1 DPA:<1 DHA:9	SDA: 21	N.A.	5	63	(Dunstan *et al*., 1992; Petrie *et al*., 2010b) (Petrie *et al*., 2010a; Ahmann *et al*., 2011)
Ostreococcus lucimarinus	DHA: 3	N.A.	7	39	(Domergue *et al*., 2005; Wagner *et al*., 2010)
Ostreococcus tauri	DHA: 12	4	59	82	

§Substrate conversion yield was calculated as described previously (Sakuradani *et al*., 2005), Conversion yield (%) = 100× ([product]/ [product + substrate]. NA: Not Available. Δ6 Des genes in these organisms were expressed in *S. cerevisiae* and the substrate preference was studied by feeding the yeast with exogenous precursor fatty acids as substrates

Table 4. Δ6 Des with no significant substrate preference

Organism	Main PUFAs (%)	n-3 FAs/n-6 FAs	Δ6 Des substrate conversion yield§ (%) LA	ALA	Reference
Parietochloris incisa	ARA: 57 EPA: <1	<1	5	5	(Iskandarov *et al*., 2010) (Wada and Murata, 1990;
Synechocystis sp. PCC 6803	GLA: 21 SDA: 8	<1	68	57	Reddy *et al*., 1993) (Tan *et al*., 2011)
Conidiobolus obscurus	GLA: 3 DGLA: 7 ARA: 10 ETA: 1 EPA: 7	<1	15	16	(O'Brien *et al*., 1993;
Pythium irregulare	ARA: 11* EPA: 14* ARA: 10†				Hong *et al*., 2002)

Table 4. Continue

	EPA: 25 †				
		1[a]			
		3[b]	80	67	(Grima *et al*., 1996;
Phaeodactylum tricornutum	ETA: <1				Domergue *et al*., 2002)
	ARA: 1				
	EPA: 36				
	DHA: 3				
		39	28	27	(Kawachi *et al*., 2002;
Glossomastix chrysoplasta	ARA: 6				Hsiao *et al*., 2007)
	EPA: 39				
	DHA: 9				
		8	6	7	
Thalassiosira pseudonana	EPA: 17				(Tonon *et al*., 2002;
	DHA: 5	N.A.	68	80	Tonon *et al*., 2005)

*Grown at 22°C,†Grown at 14°C, §Substrate conversion yield was calculated as described previously (Sakuradani *et al*., 2005), Conversion yield (%) = 100× ([product]/ [product + substrate]. Δ6 Des gene from P. irregular was expressed in Brassica Juncea, substrate preference was calculated based on its endogenous fatty acids production. Δ6 Des gene from *Synechocystis* sp. PCC 6803 was expressed in Anabaena, substrate preference was calculated based on its endogenous fatty acids production. Δ6 Des from all other organisms were expressed in *S. cerevisiae* and the substrate preference was studied by feeding the yeast with exogenous precursor fatty acids as substrates

Table 5. ω-3 Des from different species[a]

			ω-3 Des substrate conversion yield[§] (%)		
Organism	Main PUFAs (%)	n-3 FAs/n-6 FAs	18C	20C	Reference
Saprolegnia diclina	ARA: 10				(Gellerman and Schlenk, 1979;
	EPA: 20	2	0	31	Pereira *et al*., 2004)
Mortierella alpina 1S-4	ARA: 48[*]				(Shimizu *et al*., 1988;
	EPA: 0[*]				Sakuradani *et al*., 2005)
	ARA: 28[†]				
	EPA: 15[†]				
		0[a]			
		<1[b]	20	10	
Synechocystis sp. PCC 6803	GLA: 21				(Wada and Murata, 1990;
	SDA: 8	<1	25	?	Sakamoto *et al*., 1994)

*Strain was cultivated at 28°C †Strain was cultivated at 12°C §Yield of substrate conversion was calculated as described previously (Sakuradani *et al*., 2005), Conversion yield (%) = 100× ([product]/ [product + substrate]. ω-3 Des gene from *Synechocystis* sp. PCC 6803 was expressed in *Synechococcus* sp. PCC 7942. ω-3 Des genes from other organisms were expressed in *S. cerevisiae*, the substrate preference was studied by feeding the yeast with exogenous precursor fatty acids as substrates

6. SUMMARY

Most fungi, microalgae and some bacteria produce PUFAs through oxidative desaturation of fatty acids but only some of them can produce both GLA and ALA and their derived fatty acids, these organisms contain both Δ6 and ω-3 Des. The substrate preference of Δ6 Des of microorganisms plays a major role in directing the metabolic flux of LA into either n-6 or n-3 PUFAs. It produces n-6 or n-3 PUFAs as major product according to its substrate preference of n-6 or n-3 fatty acids. However when the substrate preference of Δ6 Des for LA or ALA is comparable, then the activity of ω-3 Des, which is controlled by its genetic background, but also regulated by environmental stimuli such as temperature, determines the metabolic flux of LA into n-6 or n-3 fatty acids. When the activity of ω-3 Des is very low, the microorganism produces n-6 fatty acids as major lipids; when the activity of ω-3 Des is very high, then it produces n-3 fatty acids as major lipids and at certain activity level, it may produce equal amount of n-6 and n-3 fatty acids. The substrate preference of Δ6 Des is controlled by its protein structure which is primary determined by the amino acid sequence of the protein,

whether and how this substrate preference may be regulated by other nutritional or environmental conditions is, so far, not clear. The molecular mechanism of low temperature induced expression of fatty acid desaturase in bacteria has been investigated, the two component system for the sensing of the low temperature signal and the transduction of this signal to the induction of the expression of fatty acid desaturase has been identified in some bacteria. However the mechanism for the low temperature induced expression of ω-3 Des in eukaryotic microorganisms is completely unknown. Research into this area should be exciting and provide insights to the understanding of the biochemistry of n-6/n-3 PUFA biosynthesis in oleaginous microorganisms.

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8. REFERENCES

Abd Elrazak, A., A. Ward and J. Glassey, 2013. Polyunsaturated fatty acid production by marine bacteria. Bioproc. Biosyst. Eng., 36: 1641-1652. DOI: 10.1007/s00449-013-0936-0

Aguilar, P.S., A.M. Hernandez-Arriaga, L.E. Cybulski, A.C. Erazo and D. De Mendoza *et al*., 2001. Molecular basis of thermosensing: a two-component signal transduction thermometer in Bacillus subtilis. EMBO J., 20: 1681-1691. DOI: 10.1093/emboj/20.7.1681

Ahmann, K., M. Heilmann and I. Feussner, 2011. Identification of a Δ4-desaturase from the microalga Ostreococcus lucimarinus. Eur. J. Lipid Sci. Technol., 113: 832-840. DOI: 10.1002/ejlt.201100069

Ando, A., Y. Sumida, H. Negoro, D.A. Suroto and S. Shimizu *et al*., 2009. Establishment of Agrobacterium tumefaciens-mediated transformation of an oleaginous fungus, *Mortierella alpina* 1S-4 and its application for eicosapentaenoic acid producer breeding. Applic. Environ. Microbiol., 75: 5529-5535. DOI: 10.1128/AEM.00648-09

Bajpai, P., P. Bajpai and O. Ward, 1992. Optimisation of culture conditions for production of eicosapentaenoic acid by *Mortierella* elongate NRRL 5513. J. Ind. Microbiol., 9: 11-17. DOI: 10.1007/BF01576363

Candela, C.G., L.M.B. López and V.L. Kohen, 2011. Importance of a balanced omega 6/omega 3 ratio for the maintenance of health. Nutritional recommendations. Importancia del equilibrio del índice omega-6/omega-3 en el mantenimiento de un buen estado de salud recomendaciones. Nutr. Hosp., 26: 323-329.

Domergue, F., A. Abbadi, U. Zähringer, H. Moreau and E. Heinz *et al*., 2005. In vivo characterization of the first acyl-CoA Δ6-desaturase from a member of the plant kingdom, the microalga *Ostreococcus tauri*. Biochem. J., 389: 483-483. DOI: 10.1042/BJ20050111

Domergue, F., J. Lerchl, U. Zähringer and E. Heinz, 2002. Cloning and functional characterization of Phaeodactylum tricornutum front-end desaturases involved in eicosapentaenoic acid biosynthesis. Eur. J. Biochem., 269: 4105-4113. DOI: 10.1046/j.1432-1033.2002.03104.x

Dunstan, G.A., J.K. Volkman, S.W. Jeffrey and S.M. Barrett, 1992. Biochemical composition of microalgae from the green algal classes Chlorophyceae and Prasinophyceae. 2. Lipid classes and fatty acids. J. Exp. Mar. Biol. Ecol., 161: 115-134. DOI: 1016/0022-0981(92)90193-E

Dyal, S.D. and S.S. Narine, 2005. Implications for the use of *Mortierella* fungi in the industrial production of essential fatty acids. Food Res. Int., 38: 445-467. DOI: 10.1016/j.foodres.2004.11.002

Gellerman, J.L. and H. Schlenk, 1979. Methyl-directed desaturation of arachidonic to eicosapentaenoic acid in the fungus, Saprolegnia parasitica. Biochimica et Biophysica Acta (BBA)-Lipids Lipid Metabolism, 573: 23-30. DOI: 10.1016/0005-2760(79)90169-3

Grima, E.M., A.R. Medina, A.G. Giménez and M.J.I. González, 1996. Gram-scale purification of Eicosapentaenoic Acid (EPA, 20: 5n-3) from wet Phaeodactylum tricornutum UTEX 640 biomass. J. Applic. Phycol., 8: 359-367. DOI: 10.1007/BF02178579

Gunstone, F.D., 1992. Gammar linolenic acid-occurrence and physical and chemical properties. Prog. Lipid Res., 31: 145-161. DOI: 1016/0163-7827(92)90007-6

Harris, W., 2006. The omega-6/omega-3 ratio and cardiovascular disease risk: Uses and abuses. Current Atherosclerosis Reports, 8: 453-459. DOI: 10.1007/s11883-006-0019-7

Hirano, M., H. Mori, Y. Miura, N. Matsunaga and T. Matsunaga *et al*., 1990. γ-linolenic acid production by microalgae. Applic. Biochem. Biotechnol., 24: 183-191. DOI: 10.1007/BF02920244

Hong, H., N. Datla, D.W. Reed, P.S. Covello and X. Qiu *et al*., 2002. High-level production of γ-linolenic acid in brassica juncea using a Δ6 desaturase from pythium irregulare. Plant Physiol., 129: 354-362. DOI: 10.1104/pp.001495

Hsiao, T.Y., B. Holmes and H.W. Blanch, 2007. Identification and functional analysis of a delta-6 desaturase from the marine microalga Glossomastix chrysoplasta. Mar. Biotechnol. NY, 9: 154-165. DOI: 10.1007/s10126-006-6075-8

Iskandarov, U., I. Khozin-Goldberg and Z. Cohen, 2010. Identification and Characterization of Δ12, Δ6 and Δ5 Desaturases from the Green Microalga Parietochloris incisa. Lipids, 45: 519-530. DOI: 10.1007/s11745-010-3421-4

Kawachi, M., I. Inouye, D. Honda, C.J. O'Kelly and R.A. Andersen, 2002. The pinguiophyceae classis nova, a new class of photosynthetic stramenopiles whose members produce large amounts of omega-3 fatty acids. Phycol. Res., 50: 31-47. DOI: 10.1111/j.1440-1835.2002.tb00134.x

Kennedy, M.J., S.L. Reader and R.J. Davies, 1993. Fatty acid production characteristics of fungi with particular emphasis on gamma linolenic acid production. Biotechnol. Bioeng., 42: 625-634. DOI: 10.1002/bit.260420511

Larkin, R.P. and C.L. Groves, 2003. Identification and Characterization of Isolates of Phytophthora infestans Using Fatty Acid Methyl Ester (FAME) Profiles. Plant Dis., 87: 1233-1243. DOI: 10.1094/PDIS.2003.87.10.1233

Lenihan-Geels, G., K. Bishop and L. Ferguson, 2013. Alternative sources of omega-3 fats: Can we find a sustainable substitute for fish. Nutrients, 5: 1301-1315. DOI: 10.3390/nu5041301

Liu, J., D. Li, Y. Yin, H. Wang and L. Yu *et al*., 2011. Δ6-Desaturase from *Mortierella alpina*: CDNA cloning, expression and phylogenetic analysis. Biotechnol. Lett., 33: 1985-1991. DOI: 10.1007/s10529-011-0650-4

Liu, J., M. Sommerfeld and Q. Hu, 2013. Screening and characterization of Isochrysis strains and optimization of culture conditions for docosahexaenoic acid production. Applic. Microbiol. Biotechnol., 97: 4785-4798. DOI: 10.1007/s00253-013-4749-5

Metz, J.G., P. Roessler, D. Facciotti, C. Levering and J. Browse *et al*., 2001. Production of polyunsaturated fatty acids by polyketide synthases in both prokaryotes and eukaryotes. Science, 293: 290-293. DOI: 10.1126/science.1059593

Murata, N. and H. Wada, 1995. Acyl-lipid desaturases and their importance in the tolerance and acclimatization to cold of cyanobacteria. Biochem. J., 308: 1-8. PMID: 7755550.

Nagano, N., K. Sakaguchi, Y. Taoka, Y. Okita and M. Hayashi *et al*., 2011. Detection of genes involved in fatty acid elongation and desaturation in thraustochytrid marine eukaryotes. J. Oleo Sci., 60: 475-481. DOI: 10.5650/jos.60.475

Nguyen, H.M., S., Cuiné, A. Beyly-Adriano, B. Légeret and Y. Li-Beisson *et al*., 2013. The green microalga chlamydomonas reinhardtii has a single ω-3 fatty acid desaturase that localizes to the chloroplast and impacts both plastidic and extraplastidic membrane lipids. Plant Physiol., 163: 914-928. DOI: 10.1104/pp.113.223941

Nitsan, Z., S. Mokady and A. Sukenik, 1999. Enrichment of poultry products with ω3 fatty acids by dietary supplementation with the alga nannochloropsis and mantur oil. J. Agric. Food Chem., 47: 5127-5132. DOI: 10.1021/jf981361p

O'Brien, D.J., M.J. Kurantz and R. Kwoczak, 1993. Production of eicosapentaenoic acid by the filamentous fungus Pythium irregulare. Applic. Microbiol. Biotechnol., 40: 211-214. DOI: 10.1007/BF00170368

Pereira, S.L., A.E. Leonard and P. Mukerji, 2003. Recent advances in the study of fatty acid desaturases from animals and lower eukaryotes. Prostaglandins Leukotrienes Essential Fatty Acids, 68: 97-106. DOI: 10.1016/S0952-3278(02)00259-4

Pereira, S.L., Y.S. Huang, E.G. Bobik, A.J. Kinney and P. Mukerji *et al*., 2004. A novel omega3-fatty acid desaturase involved in the biosynthesis of eicosapentaenoic acid. Biochem. J., 378: 665-671. DOI: 10.1042/BJ20031319

Petrie, J.R., Q. Liu, A.M. Mackenzie, P. Shrestha and S.P. Singh *et al*., 2010a. Isolation and characterisation of a high-efficiency desaturase and elongases from microalgae for transgenic LC-PUFA production. Mar. Biotechnol., 12: 430-438. DOI: 10.1007/s10126-009-9230-1

Petrie, J.R., P. Shrestha, M.P. Mansour, P.D. Nichols and S.P. Singh *et al*., 2010b. Metabolic engineering of omega-3 long-chain polyunsaturated fatty acids in plants using an acyl-CoA Δ6-desaturase with ω3-preference from the marine microalga Micromonas pusilla. Metab. Eng., 12: 233-240. DOI: 10.1016/j.ymben.2009.12.001

Rateldge, C., 1997. Microbial Lipids. In: Biotechnology: Products of Secondary Metabolism, Rehm, H.J., R. Reed, A. Pulher, H. Kleinkauf and H. Dohren (Eds.), VCH, Weinheim, Germany, pp: 133-197.

Reddy, A.S., M.L. Nuccio, L.M. Gross and T.L. Thomas, 1993. Isolation of a Δ6-desaturase gene from the cyanobacterium *Synechocystis* sp. strain PCC 6803 by gain-of-function expression in Anabaena sp. strain PCC 7120. Plant Mol. Biol., 22: 293-300. DOI: 10.1007/BF00014936

Sakamoto, T. and N. Murata, 2002. Regulation of the desaturation of fatty acids and its role in tolerance to cold and salt stress. Curr. Opin. Microbiol., 5: 206-210. DOI: 10.1016/S1369-5274(02)00306-5

Sakamoto, T., D.A. Los, S. Higashi, H. Wada and N. Murata *et al*., 1994. Cloning of ω3 desaturase from cyanobacteria and its use in altering the degree of membrane-lipid unsaturation. Plant Mol. Biol., 26: 249-263. DOI: 10.1007/BF00039536

Sakuradani, E. and S. Shimizu, 2003. Gene cloning and functional analysis of a second Δ6-fatty acid desaturase from an arachidonic acid-producing *Mortierella* fungus. Biosci. Biotechnol. Biochem., 67: 704-711. DOI: 10.1271/bbb.67.704

Sakuradani, E., T. Abe, K. Iguchi and S. Shimizu, 2005. A novel fungal omega3-desaturase with wide substrate specificity from arachidonic acid-producing *Mortierella alpina* 1S-4. Applic. Microbiol. Biotechnol., 66: 648-654. DOI: 10.1007/s00253-004-1760-x

Sayanova, O., J.A. Napier and P.R. Shewry, 1999. Δ6-Unsaturated fatty acids in species and tissues of the Primulaceae. Phytochemistry, 52: 419-422. DOI: 10.1016/S0031-9422(99)00256-3

Sayanova, O., R. Haslam, M. Venegas-Calerón and J. Napier, 2006. Identification of Primula “front-end” desaturases with distinct n-6 or n-3 substrate preferences. Planta, 224: 1269-1277. DOI: 10.1007/s00425-006-0306-0

Sayanova, O.V., F. Beaudoin, L.V. Michaelson, P.R. Shewry and J.A. Napier *et al*., 2003. Identification of Primula fatty acid Δ6-desaturases with n-3 substrate preferences. FEBS Lett., 542: 100-104. DOI: 10.1016/S0014-5793(03)00358-2

Scott, D.D., K. Srirama and B.S. Carani, 2007. Omega-3 fatty acids for nutrition and medicine: Considering microalgae oil as a vegetarian source of EPA and DHA. Curr. Diabetes Rev., 3: 198-203. DOI: 10.2174/157339907781368968

Seto, A., H.L. Wang and C.W. Hesseltine, 1984. Culture conditions affect eicosapentaenoic acid content of Chlorella minutissima. J. Am. Oil Chem. Soc., 61: 892-894. DOI: 10.1007/BF02542159

Shi, Y., L. An, X. Li, C. Huang and G. Chen *et al*., 2011. The octadecanoid signaling pathway participates in the chilling-induced transcription of ω-3 fatty acid desaturases in Arabidopsis. Plant Physiol. Biochem., 49: 208-215. DOI: 10.1016/j.plaphy.2010.11.013

Shimizu, S., Y. Shinmen, H. Kawashima, K. Akimoto and H. Yamada *et al*., 1988. Fungal mycelia as a novel source of eicosapentaenoic acid: Activation of enzyme(s) involved in eicosapentaenoic acid production at low temperature. Biochem. Biophys. Res. Commun., 150: 335-341. DOI: 10.1016/0006-291X(88)90525-6

Shimizu, K., K. Suhara, M. Ikumo, M.I. Eremets and K. Amaya, 1998. Superconductivity in oxygen. Nature, 393: 767-769. DOI: 10.1038/31656

Shivaji, S. and J.S. Prakash, 2010. How do bacteria sense and respond to low temperature. Arch. Microbiol., 192: 85-95. DOI: 10.1007/s00203-009-0539-y

Sun, Q., J. Liu, Q. Zhang, X. Qing and B. Qi *et al*., 2013. Characterization of three novel desaturases involved in the delta-6 desaturation pathways for polyunsaturated fatty acid biosynthesis from Phytophthora infestans. Applic. Microbiol. Biotechnol., 97: 7689-7697. DOI: 10.1007/s00253-012-4613-z

Suzuki, I. and N. Murata, 2000. Perception and transduction of low-temperature signals to induce desaturation of fatty acids. Biochem. Soc. Trans., 28: 628-630. DOI: 10.1042/BST0280628

Takemura, M., T. Hamada, H. Kida and K. Ohyama, 2012. Cold-Induced accumulation of ω-3 polyunsaturated fatty acid in a liverwort, *Marchantia polymorpha* L. Biosci Biotechnol Biochem., 76: 785-790. DOI: 10.1271/bbb.110915

Tan, L., D. Meesapyodsuk and X. Qiu, 2011. Molecular analysis ofΔ 6 desaturase andΔ 6 elongase from Conidiobolus obscurus in the biosynthesis of eicosatetraenoic acid, a ω3 fatty acid with nutraceutical potentials. Applic. Microbiol. Biotechnol., 90: 591-601. DOI: 10.1007/s00253-010-3060-y

Tonon, T., D. Harvey, T.R. Larson and I.A. Graham, 2002. Long chain polyunsaturated fatty acid production and partitioning to triacylglycerols in four microalgae. Phytochemistry, 61: 15-24. DOI: 10.1016/S0031-9422(02)00201-7

Tonon, T., O. Sayanova, L.V. Michaelson, R. Qing and I.A. Graham *et al*., 2005. Fatty acid desaturases from the microalga Thalassiosira pseudonana. FEBS J., 272: 3401-3412. DOI: 10.1111/j.1742-4658.2005.04755.x

Wada, H. and N. Murata, 1990. Temperature-induced changes in the fatty acid composition of the cyanobacterium, *Synechocystis* PCC6803. Plant Physiol., 92: 1062-1062. DOI: 10.1104/pp.92.4.1062

Wagner, M., K. Hoppe, T. Czabany, M. Heilmann and G. Daum *et al*., 2010. Identification and characterization of an acyl-CoA: Diacylglycerol Acyltransferase 2 (DGAT2) gene from the microalga O. tauri. Plant Physiol. Biochem., 48: 407-416. DOI: 10.1016/j.plaphy.2010.03.008

Wang, L., W. Chen, Y. Feng, Y. Ren and I.M. Berquin *et al*., 2011. Genome characterization of the oleaginous fungus *Mortierella alpina*. PloS One, 6: 28319-28319. DOI: 10.1371/journal.pone.0028319

Weete, J.D. and S.R. Gandhi, 1992. Industrial Applications of Single Cell Oils. In: Enhancement of C20 Polyunsaturated Fatty Acid Production in Pythium Ultimum, Kyle, D.J. and C. Ratledge (Eds.), AOCS Publishing, ISBN-10: 978-0-935315-39-4, pp: 300.

Zhang, P., S. Liu, B. Cong, G. Wu and X. Huang *et al*., 2011. A novel omega-3 fatty acid desaturase involved in acclimation processes of polar condition from Antarctic ice algae Chlamydomonas sp. ICE-L. Mar. Biotechnol., 13: 393-401. DOI: 10.1007/s10126-010-9309-8

Zhu, M., P.P. Zhou and L.J. Yu, 2002. Extraction of lipids from *Mortierella alpina* and enrichment of arachidonic acid from the fungal lipids. Bioresour. Technol., 84: 93-95. DOI: 10.1016/S0960-8524(02)00028-7