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 (PUFAs) 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 fatty 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 differential 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 Stearidonic 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 Δ3 Desaturase (Δ3 Des) is present in these organisms (Sayanova et al., 1999; 2003; 2006). However, in general, higher plants does not produce PUFA's 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 referred 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 protists, 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 oleaginous 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 production (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 Mortierella alpina, which 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/Mucic2/Mucic2.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 (Rateledge, 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; Zha 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 low temperatures (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 Conidiothecus 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 n-3 fatty acid as major lipids 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 Micromonas 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, Glossostix chrysoplata 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        | (Wada and Murata, 1990; Reddy et al., 1993; Sakamoto et al. 1994)         |
| Yeast              | *Candida diddensiae*             | ALA: 5         | (Rateldge, 1997)                                                          |
| *Mortierella*      | *Mortierella elongate*           | GLA: 37        |                                                                           |
|                    | *Pythium irregular*              | GLA: 2         |                                                                           |
| Filamentous fungus | *Pythium ultimum*                | ARA: 15        | (Weete and Gandhi, 1992)                                                  |
| Microalgae         | *Ostreococcus lucimarinus*       | DHA: 3         | (Petrie et al., 2010a; Ahmann et al., 2011)                               |
|                    | *Phaeodactylum tricornutum*      | DHA: 12        | (Domergue et al., 2005; Wagner et al., 2010)                              |
|                    | *Chlorella NKG042401*            | GLA: 11        |                                                                           |
|                    | *Thraustochytriaceae*            | DHA: 6         |                                                                           |
|                    | *Chlorella minutissima*          | EPA: 45        | (Seto et al., 1984)                                                       |
|                    | *Chlorella*                      | GLA: 11        |                                                                           |
|                    | *Nannochloropsis oculata*        | GLA: 2         |                                                                           |
|                    | *Nannochloropsis sp.*           | EPA: 38        | (Tonon et al., 2002)                                                      |
|                    | *Nannochloropsis sp.*           | ARA: 5         |                                                                           |
|                    | *Schizochytrium sp.*            | EPA: 3         |                                                                           |
|                    | *Thraustochytriaceae*           | DHA: 6         |                                                                           |
|                    | *Isochrysis galbana*             | EPA: 25        | (Liu et al., 2013)                                                        |
|                    | *Nannochloropsis oculata*        | DHA: 11        |                                                                           |
|                    | *Monodopsidaceae*               | EPA: 28        |                                                                           |
### Table 2. ∆6 Des with substrate preference of LA

| Organisms     | Main PUFAs (%) | n-3 FAs/n-6 FAs | LA | ALA | Reference                                      |
|---------------|----------------|-----------------|----|-----|------------------------------------------------|
| Mt. alpina 1S-4 | ARA: 48        |                 | LA | ALA |                   |
|                | EPA: 0*        |                 |    |     | (Shimizu et al., 1988; Sakuradani and Shimizu, 2003) |
|                | GLA: 10³       |                 |    |     |                                                |
|                | ARA: 28³       |                 | 0* |     | (Zhu et al., 2002; Liu et al., 2011)            |
|                | EPA: 15³       |                 | <1b|     |                                                |
| Mt. alpina W15 | GLA: 4         |                 | 47 | 30  |                                                |
|                | DGLA: 3        |                 |    |     |                                                |
|                | AA: 9          |                 |    |     |                                                |

*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 (%) = 100x ([product]/ [product + substrate]. ∆6 Desaturase gene from M. alpina 1S-4 was expressed in A. oryzae. ∆6 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. ∆6 Des with substrate preference of ALA

| Organisms                  | Main PUFAs (%) | n-3 FAs/n-6 FAs | LA | ALA | Reference                                      |
|----------------------------|----------------|-----------------|----|-----|------------------------------------------------|
| Micromonas pusilla         | SDA: 21        |                 |    |     | (Dunstan et al., 1992; Petrie et al., 2010b)   |
| EPA:<1                     |                |                 |    |     | (Petrie et al., 2010a; Ahmann et al., 2011)    |
| DPA:<1                     |                |                 |    |     | (Domergue et al., 2005; Wagner et al., 2010)   |
| Osteococcus lucimarinus    | DHA: 3         | N.A.            | 5  | 63  |                                                |
| Osteococcus tauri          | DHA: 12        |                 | 4  | 59  | 82                                            |

§Substrate conversion yield was calculated as described previously (Sakuradani et al., 2005). Conversion yield (%) = 100x ([product]/ [product + substrate]. NA: Not Available. ∆6 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. ∆6 Des with no significant substrate preference

| Organism                    | Main PUFAs (%) | n-3 FAs/n-6 FAs | LA | ALA | Reference                                      |
|-----------------------------|----------------|-----------------|----|-----|------------------------------------------------|
| Parietochloris incisa       | ARA: 57        |                 |    |     | (Iskandarov et al., 2010)                       |
| EPA:<1                      |                |                 |    |     | (Wada and Murata, 1990; Reddy et al., 1993)    |
| Synechocystis sp. PCC 6803  | GLA: 21        |                 | <1 | 5  | 5                                            |
| SDA: 8                      |                |                 |    |     | (Tan et al., 2011)                             |
| Conidiobolus obscurus       | GLA: 3         |                 | <1 | 68  | 57                                           |
| DGLA: 7                     |                |                 |    |     | (O’Brien et al., 1993; Hong et al., 2002)      |
| ARA: 10                     |                |                 |    |     |                                               |
| ETA: 1                      |                |                 |    |     |                                               |
| EPA: 7                      |                |                 |    |     |                                               |
| Pythium irregularare        | ARA: 11³       |                 | <1 | 15  | 16                                           |
| EPA: 14²                    |                |                 |    |     |                                               |
| ARA: 10³                    |                |                 |    |     |                                               |
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Table 4. Continue

| Organism                          | EPA: 25† | ARA: 1 | EPA: 36 | DHA: 3 | EPA: 39 | DHA: 9 | EPA: 17 | DHA: 5 | 1* | 80 | 67 | (Grima et al., 1996; Domergue et al., 2002) |
|-----------------------------------|----------|--------|---------|--------|---------|--------|---------|--------|-----|-----|-----|---------------------------------------------|
| Phaeodactylum tricornutum         | ETA: <1  |        |         |        |         |        |         |        | 3b  |     |     | (Kawachi et al., 2002; Hsiao et al., 2007)  |
|                                   | ARA: 6   | EPA: 39| DHA: 9  |        |         |        |         |        | 8b  | 6   | 7  |                                             |
| Glossomastix chrysoplasla         | ARA: 6   | EPA: 39| DHA: 9  |        |         |        |         |        | 8b  | 6   | 7  |                                             |
| Thalassiosira pseudonana          | EPA: 17  |        |         |        |         |        |         |        | 8b  | 6   | 7  | (Tonon et al., 2002; 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]). ∆6 Des gene from P. irregular was expressed in Brassica Juncea, substrate preference was calculated based on its endogenous fatty acids production. ∆6 Des gene from Synechocystis sp. PCC 6803 was expressed in Anabaena, substrate preference was calculated based on its endogenous fatty acids production. ∆6 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 speciesa

| Organism              | Main PUFAs (%) | n-3 FAs/n-6 FAs | ω-3 Des substrate conversion yield (%) | Reference                                      |
|-----------------------|----------------|-----------------|--------------------------------------|------------------------------------------------|
| Saprolegnia diclina   | ARA: 10        | EPA: 20         | 2                                    | 0 31                                          | (Gellerman and Schlenk, 1979; Pereira et al., 2004) |
| Mortierella alpina 1S-4| ARA: 48†       | EPA: 0†         | 0†                                   | 31                                           | (Shimizu et al., 1988; Sakuradani et al., 2005)   |
|                       | ARA: 28†       | EPA: 15†        | <1†                                  | 20 10                                        |                                                 |
| Synechocystis sp. PCC 6803 | GLA: 21      | SDA: 8          | 0†                                   | 20 10                                        | (Wada and Murata, 1990; 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 ∆6 and ω-3 Des. The substrate preference of ∆6 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 ∆6 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 ∆6 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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