Journal of Bioscience and Bioengineering
VOL. 116 No. 4, 417–422, 2013

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REVIEW

Metabolic engineering for the production of polyunsaturated fatty acids by oleaginous fungus *Mortierella alpina* 1S-4

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Received 8 March 2013; accepted 3 April 2013
Available online 3 May 2013

Researches related with the application of functional lipids such as polyunsaturated fatty acids (PUFAs) have been conducted in various fields with a view to health and dietary requirements. Novel rich sources other than known natural sources such as plant seeds and fish oils are required for increasing demands of PUFAs. The filamentous fungus *Mortierella alpina* 1S-4 produces triacylglycerols rich in arachidonic acid, i.e., ones reaching 20 g/l in concentration and containing 30–70% arachidonic acid as total fatty acids. Various mutants derived from *M. alpina* 1S-4 have led to the production of oils containing various PUFAs. Molecular breeding of *M. alpina* strains by means of manipulation of the genes involved in PUFA biosynthesis facilitates improvement of PUFA productivity and elucidation of the functions of their enzymes. This review describes practical PUFA production through mutant breeding, functional analyses of the genes of the enzymes involved in PUFA biosynthesis, and recent advances in unique PUFA production through molecular breeding.

[**Key words:** *Mortierella alpina*; Polyunsaturated fatty acid; Arachidonic acid (AA); Eicosapentaenoic acid (EPA); Molecular breeding; Fatty acid desaturase]

Polyunsaturated fatty acids (PUFAs) contain more than one double bond, and some 20-carbon (C20) PUFAs play important roles not only as structural components of membrane phospholipids but also as precursors of eicosanoids, signaling molecules including prostaglandins, thromboxanes, and leukotrienes, that are essential for all mammals. Especially, arachidonic acid (AA; 20:4n-6), a representative n-6 PUFA, is the most abundant C20 PUFA in humans, and not only exhibits various regulation effects and physiological activities but also plays important roles in infant nutrition (1,2). Eicosapentaenoic acid (EPA; 20:5n-3), a representative n-3 PUFA, is beneficial in the treatment of cardiovascular diseases (3), and decreases platelet aggregation and blood pressure (4). The distinct functions of the two families make the ratio in the diet of n-6 and n-3 PUFAs important in inflammatory responses and cardiovascular health. The most readily available lipid sources relatively rich in C20 PUFAs, none of which are found in plants, are fish oils, animal tissues, and algal cells. Transgenic plants with some exogenous desaturase genes have been reported to produce n-3 and n-6 PUFAs (5). However, these transgenic sources are unsuitable for practical purposes from the viewpoint of genetically modified organisms. The term "single cell oils" is used for unique oils produced by microorganisms that compete with plant-seed oils and fish oils (6). Some yeasts and molds are known as microorganisms that accumulate high levels of triacylglycerols. A lipid content in excess of 40% (w/w) is not exceptional, and values of 70% and even 80% have been reported (7). Single cell oils having different fatty acid compositions from plant-seed oils and fish oils are valuable for human life.

On screening of the microorganisms accumulating C20 PUFAs, a filamentous fungus, *Mortierella alpina* 1S-4, was isolated as a suitable source for the AA production; it was able to produce EPA through the n-3 PUFA biosynthetic pathway, while AA through the n-6 PUFA biosynthetic pathway (8–10). In this strain, most PUFAs are present in triacylglycerols as storage oils, while some are present in phospholipids as structural components of membranes.

Although success in this area over the last 25 years has generated much interest in the development of microbial fermentation processes, manipulation of the lipid compositions of microorganisms requires new biotechnological strategies to obtain high yields of the desired PUFAs. This article reviews recent progress in the breeding of commercially important arachidonic acid-producing *M. alpina* strains, particularly approaches to creating desaturase and elongase mutants with unique pathways for PUFA biosynthesis involving conventional chemical mutagenesis and modern molecular genetics.

VARIOUS KINDS OF PUFAS IN *M. ALPINA* 1S-4

Isolation of mutants producing PUFAs through different biosynthetic pathways Various mutants defective in desaturase (Δ9, Δ12, Δ6, Δ5 and ω3) or elongase (MALCE1) activities, or

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1389-1723/$ – see front matter
http://dx.doi.org/10.1016/j.jbiosc.2013.04.008

TABLE 1. Lipid productivities of *Mortierella alpina* 1S-4 mutants.

Deficient enzyme	Mutation site	Parent strain	Mutant	Accumulation	Reference
Δ9	G265D	1S-4	T4	18:0 (40%)	13
Δ12	P166L	1S-4, Mut48,[a] and M209-7[a]	JT-180	MA (2.6 g/l, 49% of total fatty acids) enhanced activities of Δ5 and Δ6 desaturases	14
Δ12 and Δ5	P166L in (Δ12) and W301Stop (Δ5)	1S-4 and Mut48[a]	M226-9	20:2n-9 (2.2 g/l, 37%)	15
Δ6	Incorrect splicing	1S-4	Mut49	20:3n-6(Δ5) (0.48 g/l, 7%)	16
Δ5	Incorrect splicing	1S-4	S14	DGLA (4.1 g/l, 42%) and AA content (<1%)	17
ω3	W232Stop	1S-4	Y11	AA (1.5 g/l, 45%) without n-3 PUFAs	18
EL1	H154Y andT185I	1S-4	M1	16:0 (30%), 16:1n-7 (8%), and n-4/n-7 PUFAs (30%)	–
N.D.[b]	–	1S-4	KY1	Diacylglycerol (30% of total lipids)	–
N.D.	–	1S-4	V6	Lipid excretion (10–40% of total lipids)	–

[a] Mutants derived from *M. alpina* 1S-4.
[b] N.D., not determined.

with enhanced desaturase activities (Δ6 and Δ5) have been derived from *M. alpina* 1S-4 by treating the parental spores with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (11). In addition, a diacylglycerol-accumulating mutant and several lipid-excretive ones have been obtained by the same method. They are valuable not only as producers of useful PUFAs (novel or already existing) but also for providing valuable information on PUFA biosynthesis in this fungus (12). The main features of these mutants are summarized in Table 1.

Δ9 Desaturase-defective mutants accumulate stearic acid (18:0) as the main fatty acid (up to 40%) in the mycelial oil (13). Δ12 Desaturase-defective mutants accumulate high levels of n-9 PUFAs, such as Mead acid (MA; 20:3n-9) that are not detected in the wild strain because of a complete deficiency of Δ12 desaturation (Fig. 1A). One of these mutants, JT-180, yields a large amount of MA (2.6 g/l, 49% in oil) on commercial production due to its enhanced Δ5 and Δ6 desaturase activities, not including n-6 and n-3 PUFAs (14). Double mutants defective in both Δ12 and Δ5 desaturase activities accumulate n-9 eicosadienoic acid (20:2n-9) as a final product of n-9 PUFAs in large quantities (15). Δ6 Desaturase-defective mutants accumulate linoleic acid (18:2n-6) as the main fatty acid (up to 32%) in the mycelial oil (16). These mutants are characterized by the accumulation of n-6 eicosadienoic acid (20:2n-6) and nonmethylene-interrupted n-6 eicosatrienoic acid (20:3n-6Δ5) synthesized from linoleic acid, as shown in Fig. 1B. Δ5 Desaturase-defective mutants exhibit a high dihomo-γ-linolenic acid (DGLA; 20:3n-6) level (4.1 g/l, 42% in oil) and a reduced concentration (<1%) of AA (17). One of these mutants, S14, is used for the commercial production of DGLA. ω3 Desaturase-defective mutants are unable to synthesize n-3 PUFAs at temperatures below 20°C (18), although the wild strain accumulates n-3 PUFAs such as EPA below that temperature. Therefore, these ω3-desaturase defective mutants are superior to the wild strain for lipid production with a relatively high content of AA. The fatty acid profile of elongase (EL1 for the conversion of palmitic acid, 16:0, to 18:0)-defective mutants is characterized by high levels of 16:0 and

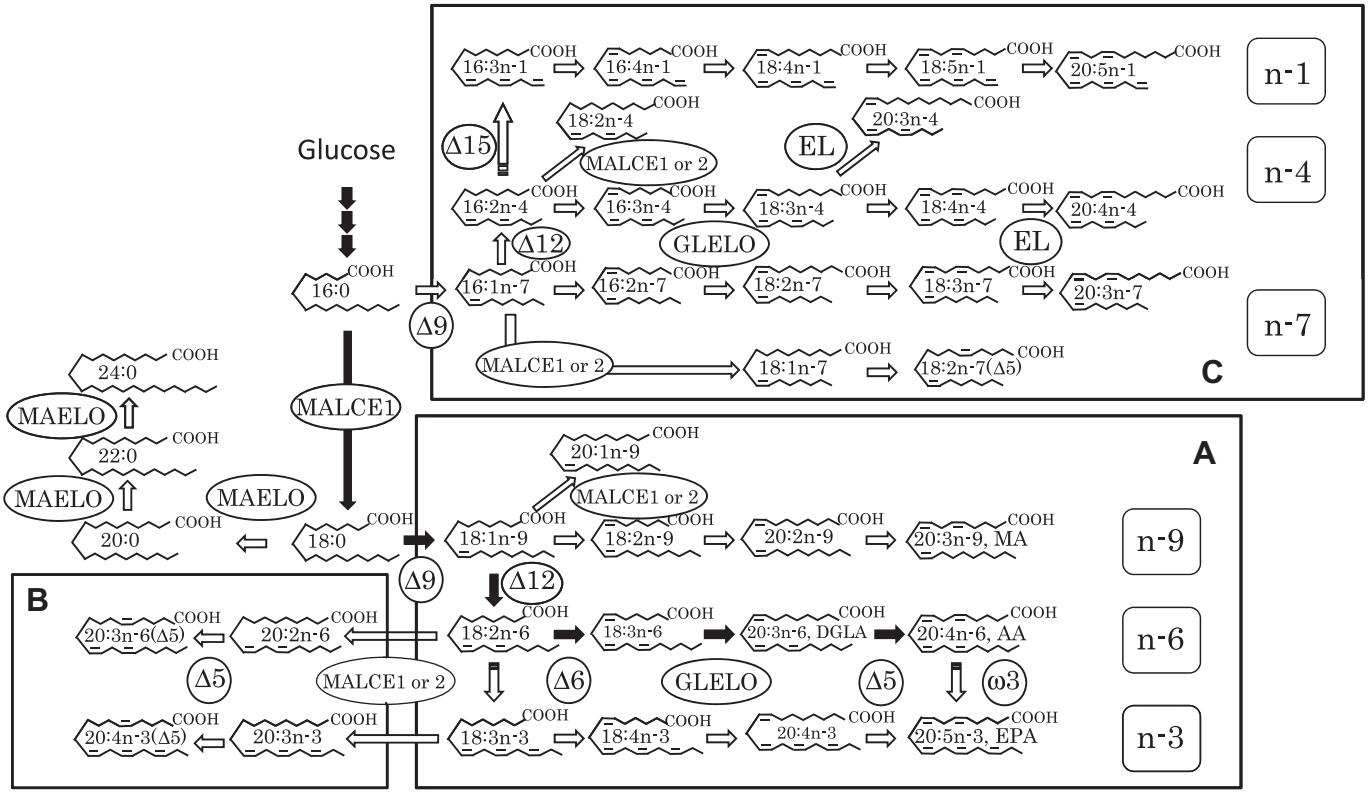

FIG. 1. Pathways for the biosynthesis of PUFAs in *M. alpina* 1S-4 and its mutants. The n-3, n-6, and n-9 PUFAs are derived from 18:1n-9 (A), the nonmethylene-interrupted PUFAs are detected in Δ6 desaturase-defective mutants (B), and the n-1, n-4, and n-7 PUFAs are derived from 16:1n-7 (C). Black arrows show the AA biosynthetic pathway in the parental strain, *M. alpina* 1S-4. AA, arachidonic acid; ΔN, ΔN desaturase; DGLA, dihomo-γ-linolenic acid; EL, fatty acid elongase; EPA, eicosapentaenoic acid; MA, Mead acid; ω3, ω3 desaturase.