Review

Arachidonic acid production by the oleaginous fungus Mortierella alpina 1S-4: A review

Hiroshi Kikukawa a,b, Eiji Sakurada a,c, Akinori Ando a, Sakayu Shimizu a,d, Jun Ogawa a,*

*Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kitashirakawa-oiwakecho, Sakyo-ku, Kyoto 606-8502, Japan
**Department of Chemistry and Biomolecular Science, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
†Institute of Technology and Science, The University of Tokushima, 2-1 Minami-Josanjima, Tokushima 770-8506, Japan
‡Department of Bioscience and Biotechnology, Faculty of Bioenvironmental Science, Kyoto Gakuen University, 1-1 Nanjo, Sogabe, Kameoka 621-8555, Japan

Abstract

The filamentous fungus Mortierella alpina 1S-4 is capable of accumulating a large amount of triacylglycerol containing C20 polyunsaturated fatty acids (PUFAs). Indeed, triacylglycerol production by M. alpina 1S-4 can reach 20 g/L of culture broth, and the critical cellular signaling and structural PUFA arachidonic acid (ARA) comprises 30%–70% of the total fatty acid. The demonstrated health benefits of functional PUFAs have in turn encouraged the search for rich sources of these compounds, including fungal strains showing enhanced production of specific PUFAs. Screening for mutants and targeted gene manipulation of M. alpina 1S-4 have elucidated the functions of various enzymes involved in PUFA biosynthesis and established lines with improved PUFA productivity. In some cases, these strains have been used for industrial-scale production of PUFAs, including ARA. In this review, we described practical ARA production through mutant breeding, functional analyses of genes encoding enzymes involved in PUFA biosynthesis, and recent advances in the production of specific PUFAs through molecular breeding of M. alpina 1S-4.

Introduction

Fatty acids containing more than one carbon double bond, termed polyunsaturated fatty acids (PUFAs), are critical sources of metabolic energy, major structural components of membrane
phospholipids, and precursors of the eicosanoid signaling mole-
cules prostaglandins, thromboxanes, and leukotrienes. Fish oils, animal fats, and algal cells are among the most readily available lipid sources rich in 20-carbon (C20) PUFAs. Among PUFAs, ARA (ARA, C20:4n-6) is the most abundant C20 PUFA in humans, espe-
cially in the brain, muscles, and liver. ARA has multiple physiologi-
cal functions and is an important nutrient for infants and the elderly [1,2]. ARA-derived lipid mediators can play various roles in establishing homeostasis for the humans [3]. However, most of the ARA in the humans is usually taken from dietary animal sources such as meat and eggs [4], and the PUFA contents of these conventional sources are insufficient for practical large-scale pro-
duction. Alternatively, γ-linolenic acid (GLA, 18:3n-6)-containing oils have been produced using Mucor fungi as the first attempt at microbial PUFA production [5,6]. Mortierella fungi, such as M. alpina ATCC32222 [7], were found as ARA producer and have been used as commercial ARA producers. Recently, the various innovations on metabolic engineering using gene engineering and meta-
obolomics for PUFA production by Mortierella fungi have reported, e.g. overexpression of malic enzyme increased the fatty acid content in M. alpina ATCC32222 [8–11].

The oil-producing filamentous fungus M. alpina 1S-4 is also a promising source of PUFA as such ARA. M. alpina 1S-4 is the first strain found as the high ARA producer and can accumulate various PUFAs through the n-6 PUFA biosynthetic pathway as well as eicosapentaenoic acid (EPA, 20:5n-3) through the n-3 PUFA biosynthetic pathway [12–14]. In M. alpina 1S-4, most PUFAs are stored in lipid droplets as triacylglycerols, while some are present in the form of phospholipids as structural components of membranes. Given the high ARA content of M. alpina 1S-4, this fungus is one of the fungal models for both fundamental and applicative studies on fatty acid biosynthesis, including the development of strains suitable for large-scale production of specific PUFAs. In fact, ARA, dihomo-γ-linolenic acid (DGLA, 20:3n-6), and Mead acid (MA, 20:3n-9) have been commercially produced by Mortierella fungi [15–19].

Although such successes over the last 30 years have generated much interest in the development of microbial fermentation pro-
cesses for the large-scale production of specific PUFAs, improved yields require more efficient biotechnological strategies for meta-
obolic engineering of microorganism lipogenesis. This article reviews recent advances in the breeding of commercially viable PUFA-producing M. alpina strains by conventional chemical muta-
genesis, the development of gene manipulation systems for M. alpina 1S-4, and the latest molecular breeding strategies for pro-
ducing rare fatty acids using molecular genetics.

**ARA-producing Mortierella sp.**

Since the first reports of Mortierella strains producing ARA in 1987 [14,20], this genus has been studied extensively as a promising single-cell oil (SCO) source for various types of PUFAs [21,22].

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>ARA productivity</th>
<th>Scale</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortierella alpina 1S-4</td>
<td>3.6 g/L/7 days</td>
<td>5 L fermentor</td>
<td>[29]</td>
</tr>
<tr>
<td></td>
<td>3.0 g/L/10 days</td>
<td>2 L fermentor</td>
<td>[12]</td>
</tr>
<tr>
<td></td>
<td>13 g/L/10 days</td>
<td>15 L fermentor</td>
<td>[25]</td>
</tr>
<tr>
<td>M. alpina ATCC32222</td>
<td>11 g/L/16 days</td>
<td>500 L fermentor</td>
<td>[28]</td>
</tr>
<tr>
<td>M. alpina ATCC32222</td>
<td>11 g/L/11 days</td>
<td>200 mL flask</td>
<td>[7]</td>
</tr>
<tr>
<td>M. alpina DSA-12</td>
<td>18.8 g/L/12.5 days</td>
<td>12 L fermentor</td>
<td>[26]</td>
</tr>
<tr>
<td>M. alpina ME-1</td>
<td>19.8 g/L/7 days</td>
<td>5 L fermentor</td>
<td>[27]</td>
</tr>
<tr>
<td>Mortierella elongata 1S-5</td>
<td>1.0 g/L/4 days</td>
<td>500 mL flask</td>
<td>[14]</td>
</tr>
<tr>
<td>Mortierella schmuckeri S12</td>
<td>2.3 g/L/3 days</td>
<td>14 L fermentor</td>
<td>[24]</td>
</tr>
<tr>
<td>Mortierella allacea YN-15</td>
<td>7.1 g/L/6 days</td>
<td>50 L fermentor</td>
<td>[23]</td>
</tr>
</tbody>
</table>

In particular, M. alpina 1S-4 has been studied for fundamental and applicative purposes, and has been used successfully for the commercial production of ARA-enriched SCO (Table 1) [7,13,14,23–29]. Mortierella alpina 1S-4 has the unique capability to synthesize a wide range of PUFAs (Fig. 1), and has several additional advantages as both a model organism for studies on fun-
gal lipid metabolism and an industrial lipid producer demonstrating particularly high yields of multiple PUFAs under energetically favorable culture conditions.

The total lipid fraction of M. alpina 1S-4 contains n-9, n-6, and n-3 PUFAs. The predominant PUFA, ARA, is synthesized from 16:0 by four desaturases and two elongases. Under culture conditions optimal for large-scale production, the total amount of lipid can reach 500–600 mg/g dry cell weight or 20 g/L of culture broth. Moreover, the ARA composition ranges from 30% to 70% of the total cellular fatty acid (70%–90% of which is present in triacylglycerols) [25,30,31]. This strain also produces EPA (approximately 10% of total fatty acids) with cultivation below 20 °C and exhibits higher EPA production upon the addition of α-linolenic acid (18:3n-3)-containing oils, such as linseed oil, to the medium [32].

**Enzymes involved in ARA biosynthesis in M. alpina 1S-4**

Arachidonic acid biosynthesis requires the activity of several fatty acid desaturases and elongases. The primary substrate hexade-
canoic acid (16:0) is converted to ARA in sequential steps cat-
alysed by elongase 1 (MALCE1), Δ9 desaturase, Δ12 desaturase, Δ6 desaturase, elongase 2 (GELEO), and Δ5 desaturase, re-
spectively (Fig. 1 and Table 1). Some of these enzymatic steps in M. alpina 1S-4 contain a NADH-cytochrome b5 reductase and cyto-
chrome b5 as an electron transport system for fatty acid desatura-
tion [33–35]. Cytochrome b5 is a small hemoprotein which is an integral component of the microsomal membranes and functions as an electron carrier in a number of microosomal oxidation/reduc-
tion reactions, including fatty acid desaturation, cholesterol biosynthesis and reduction of cytochrome P450.

The two Δ9 desaturase homologues (designated as Δ9-1 and Δ9-2) in M. alpina 1S-4 have a cytochrome b5-like domain linked to the carboxyl terminus, similar to yeast Δ9 desaturase [36]. The M. alpina 1S-4 Δ9-1 exhibits 45% amino acid sequence similarity with the yeast Saccharomyces cerevisiae homologue and 34% with the rat homologue, suggesting that M. alpina Δ9-1 is a con-
served membrane-bound protein using acyl-CoA as substrate. Both Δ9-1 and Δ9-2 desaturate 18:0 to oleic acid (18:1n-9). Although the Δ9-2 gene is not transcribed in the wild-type, Δ9-2 protein was expressed and exhibited Δ9 desaturation activity in a Δ9-1 gene-defective mutant [37]. The M. alpina Δ12 and o3 desaturases, both of which lack a cytochrome b5-like domain, have been charac-
terized by heterologous gene expression systems. The M. alpina Δ12 desaturation was confirmed to catalyze the desaturation of 18:1n-9 to 18:2n-6 in both S. cerevisiae and Aspergillus oryzae [38]. The M. alpina o3 desaturase shows 51% sequence identity with M. alpina Δ12 desaturase. It converts n-6 PUFAs to C18 and C20 chain lengths, and is particularly efficient at con-
verting ARA to EPA [39]. Furthermore, the M. alpina o3 desaturase exhibits two additional activities when expressed in S. cerevisiae, insertion of C=C double bonds at the Δ12-position and Δ15-position of hexadecenoic acid (16:1n-7) [40].

The M. alpina Δ5 and Δ6 desaturases have a cytochrome b5-like domain linked to the N-terminus. A complementary DNA (cDNA) encoding Δ5 desaturase has been isolated from two M. alpina strains, CBS210.32 and ATCC32221 [41,42]. Mortierella alpina Δ5 desaturase inserts C=C double bond at the Δ5-position of PUFAs, thereby converting DGLA into ARA. Two Δ6 desaturase homologues (designated Δ6-1 and Δ6-2) are also present in M. alpina
1S-4 [43,44]. Expression of the full-length cDNA clone in A. oryzae resulted in greater accumulation of GLA, reaching 25.2% of the total fatty acid content. The amino acid sequence homology between Δ6-1 and Δ6-2 is very high (92%). Usually, Δ6-1 gene transcription is 2-fold to 17-fold higher than Δ6-2 gene transcription in M. alpina 1S-4. However, transcription of the Δ6-2 gene was enhanced up to 6-2 gene transcription in M. alpina 1S-4 [52,53]. A high concentration of Zeocin (20 mg/mL)

**Gene manipulation in M. alpina 1S-4**

A transformation system for M. alpina 1S-4 has been developed using M. alpina uracil auxotrophs as the host strain and a complementary gene as a selection marker [48]. Transformation with M. alpina 1S-4 spores and a vector containing the M. alpina 1S-4 ura5 gene as a marker was achieved with high efficiency (transformation frequency of 0.4/mg of vector DNA) using microprojectile bombardment [49,50]. Southern blot analysis revealed that most of the integrated plasmids in stable transformants were present as multiple copies at ribosomal DNA (rDNA) positions and/or at random positions in the chromosomal DNA. An Agrobacterium tumefaciens-mediated transformation system for M. alpina 1S-4 has also been developed [51] in which the ura5 gene is used as a selectable marker under control of the homologous histone H4.1 promoter in the transfer-DNA region. The frequency of transformation reached more than 400/10^8 spores using this system, and Southern blot analysis revealed that most of the integrated transfer-DNAs appeared as a single copy at random position in the chromosomal DNA.

Mortierella alpina 1S-4 exhibits resistance to various antibiotics used to destroy other filamentous fungi. However, Zeocin- and Carboxin-resistance markers have been developed for selection of M. alpina 1S-4 [52,53]. A high concentration of Zeocin (20 mg/mL)

**Table 2**

Substrates and products of enzymes involved in arachidonic acid (ARA) biosynthesis in M. alpina 1S-4.

<table>
<thead>
<tr>
<th>Type</th>
<th>Isozyme</th>
<th>Substrate</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ9 desaturase</td>
<td>Δ9-1</td>
<td>18:0</td>
<td>18:1n-9</td>
</tr>
<tr>
<td></td>
<td>Δ9-2</td>
<td>18:0</td>
<td>18:1n-9</td>
</tr>
<tr>
<td>Δ12 desaturase</td>
<td>–</td>
<td>18:1n-9</td>
<td>18:2n-6</td>
</tr>
<tr>
<td>Δ6 desaturase</td>
<td>Δ6-1</td>
<td>18:2n-6</td>
<td>GLA</td>
</tr>
<tr>
<td></td>
<td>Δ6-2</td>
<td>18:2n-6</td>
<td>GLA</td>
</tr>
<tr>
<td>Δ5 desaturase</td>
<td>–</td>
<td>DGLA</td>
<td>ARA</td>
</tr>
<tr>
<td>α3 desaturase</td>
<td>–</td>
<td>n-6 PUFAs</td>
<td>n-3 PUFAs</td>
</tr>
<tr>
<td>MALCE1</td>
<td>–</td>
<td>16:1n-7</td>
<td>16:2n-4, 16:3n-1</td>
</tr>
<tr>
<td>GLELO</td>
<td>–</td>
<td>GLA</td>
<td>DGLA</td>
</tr>
<tr>
<td>Cyt.b1 reductase</td>
<td>Cyt.b1 reductase-1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Cyt.b1 reductase-2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cyt.b2</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**Fig. 1.** Biosynthetic pathway of PUFAs in Mortierella alpina 1S-4. ARA is biosynthesized through desaturation by Δ9, Δ12, Δ6, and Δ5 desaturases and elongation by MALCE1 and GLELO. The n-3, n-6, and n-9 PUFAs derived from 18:1n-9 (a), the n-1, n-4, and n-7 PUFAs derived from 16:1n-7 (b), and the non-methylene-interrupted PUFAs detected in Δ6 desaturase-defective mutants (c). ΔX, ΔX desaturase; α3, α3 desaturase; EL, fatty acid elongase; ETA, arachidonic acid; DGLA, dihomο-γ-linolenic acid; EPA, eicosapentaenoic acid; ETA, α3 eicosatetraenoic acid; MA, Mead acid.
Mutants described in the present review.

18

Numerous desaturase-deficient and/or elongase-deficient mutants have been isolated by treating *M. alpina* 1S-4 spores with the chemical mutagen N-methyl-N-nitro-N-nitrosoguanidine (Table 3) [60–65]. The *M. alpina* 1S-4 wild-type can accumulate n-3 PUFAs only when cultivated at low temperature (below 20 °C), while the o3 desaturase-defective mutants are unable to synthesize n-3 PUFAs even when grown at low temperature [60,66]. The wild-type usually shows the highest ARA yield at 20 °C, although a portion of the accumulated ARA is further converted to EPA, so the resultant oil includes a small amount of EPA (ca. 3%). Therefore, these mutants (e.g., Y11 and Y61 strain) are superior to the wild-type for production of SCO with a relatively higher ARA content [64,66]. Additionally, rare fatty acids accumulated in *M. alpina* 1S-4 by suppression of MALCE1-mediated 16:0 elongation to 18:0 or by supplementation of exogenous fatty acids such as 16:1n-7 into the culture medium (Fig. 1b).

This practical transformation system for *M. alpina* 1S-4 allows overexpression, RNA interference (RNAi), and disruption of genes involved in PUFA biosynthesis for improved production of desired PUFAs. Several valuable *M. alpina* mutants were directly transformed with drug resistance markers, or their uracil auxotrophs were transformed with the *ura5* marker. Molecular breeding of *M. alpina* 1S-4 and its mutants yielded unique fatty acid profiles and high productivities of valuable PUFAs (Table 3 and 4). Mutant JT-180 exhibits no Δ12 desaturase activity and enhanced Δ5 and tone H4.1 promoter and evaluated for expression activity. Seven promoters with high-level constitutive or time-dependent expression were selected, and deletion analysis determined the promoter regions required to retain the expression activities. Furthermore, using an inducible GAL10 promoter, an approximately 50-fold increase in GUS activity was achieved by addition of galactose to the culture media at any cultivation phase [55].

The integration of exogenous DNA into chromosomes occurs through two DNA double-strand break repair pathways, homologous recombination (HR) and non-homologous end joining (NHEJ) [56]. In HR, exogenous DNA is integrated into the chromosome using homologous regions as templates for precise gene insertion. The HR method is used frequently for insertion of exogenous expression constructs to disrupt target genes (gene targeting) (Fig. 2A). However, these two pathways are independent of one another and often function competitively [57]. Gene targeting systems have also been developed by disruption of key proteins involved in NHEJ [58,59], such as Ku80 or DNA ligase IV (lig4). We identified and disrupted the *ku80* and *lig4* genes in *M. alpina* 1S-4 to improve gene-targeting efficiency. These gene-disrupted strains showed no defect in vegetative growth, spore formation, or fatty acid production. Importantly, the efficiency of gene-targeting through HR was improved only in the *lig4*-disrupted strain, where it was 21-fold (67%) greater than that of the host strain. Metabolic engineering using *lig4* gene-disrupted strains as hosts is expected to produce higher levels of rare and beneficial PUFAs and contribute to basic research on fungal lipogenesis.

### Table 3

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Deficient enzyme</th>
<th>Product</th>
<th>Productivity and characteristics</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y11</td>
<td>o3 desaturase</td>
<td>ARA</td>
<td>1.5 g/L, 45% of total fatty acid with no n-3 PUFAs</td>
<td>[64,66]</td>
</tr>
<tr>
<td>Y61</td>
<td>o3 desaturase</td>
<td>ARA</td>
<td>1.8 g/L</td>
<td>[66]</td>
</tr>
<tr>
<td>JT-180</td>
<td>Δ12 desaturase</td>
<td>MA</td>
<td>2.6 g/L, 49%</td>
<td>[65]</td>
</tr>
<tr>
<td>S14</td>
<td>Δ5 desaturase</td>
<td>DGLA</td>
<td>4.1 g/L and low ARA content (&lt;1%)</td>
<td>[61]</td>
</tr>
</tbody>
</table>

Fig. 2. Gene-disruption through double crossing-over HR (A) and chromatograms of fatty acid methyl esters prepared from a control strain (*lig4* disruptant) and Δ5 desaturase gene-disrupted strain (B).
Δ6 desaturase activities, resulting in the efficient production of Mead acid (MA, 20:3n-9) [65]. With overexpression of the endogenous Δ12 desaturase gene, JT-180 accumulated a larger amount of ARA (2.0 g/L/7 days, 39% of total fatty acids) but little MA compared to the wild-type (1.2 g/L/7 days, 21%) due to enhanced Δ5 and Δ6 desaturation. Overexpression of the endogenous malce1 gene in M. alpina 1S-4 also led to faster and greater ARA accumulation (0.76 g/L/6 days, 34%) than in the wild-type (0.68 g/L/6 days, 28%). In addition, overexpression of the gene encoding GLELO, which has been suggested to catalyze the rate limiting step in ARA biosynthesis [67], was successfully performed in M. alpina 1S-4 [68]. The resulting transformants yielded more ARA (3.6 g/L/10 days, 28%) than the wild-type (1.9 g/L/10 days, 19%). Overexpression of both malce1 and glelo genes had substantial effects on ARA production by M. alpina 1S-4. The exogenous Δ5 and Δ6 desaturases (Pav.A5, Ost.A6) from the microalgae Pavlova salina and Ostreococcus lucimarinus and the Δ12 desaturase (Tri.A12) from the betelie Tribolium castaneum have desaturase activities for fatty acyl-CoA substrates. On the other hand, the homologous desaturases from M. alpina use phospholipids as substrates. By expressing these exogenous desaturases, higher ARA yields were obtained (unpublished data) [69]. For instance, overexpression of the Pav.A5 gene in the wild-type led to a markedly high ARA/DGLA ratio, while overexpression of the Ost.A6 gene in the wild-type led to higher 18:3n-6, DGLA, and ARA contents as proportions of total fatty acid compared to the wild-type. Similarly, overexpression of the Tri.A12 gene in the wild-type led to greater proportions of 18:2n-6, 18:3n-6, DGLA, and ARA compared to the wild-type.

The RNAi method using double-strand RNA has been applied to silence gene expression in M. alpina 1S-4 [70]. By suppressing endogenous Δ6-1 gene expression by RNAi in the mutant JT-180, 18:1n-9 accumulation reached 68.0% of total fatty acid content, and 18:1n-9 production in broth reached 2.76 g/L [45].

Overexpression systems using promoters that exhibit high transcriptional activities may facilitate further improvements in PUFA production. Usually, M. alpina can express Ổ3 desaturation activity and accumulate n-3 PUFAs when cultured at low temperatures (below 20 °C), with an EPA ratio of approximately 10%, while no accumulation of n-3 PUFAs was observed at 28 °C. However, overexpression of the endogenous Ổ3 desaturase gene in M. alpina 1S-4 at 20 °C increased EPA accumulation to 40% of total fatty acid [51]. Expression of the heterologous Saprolegnia diclina Δ17 desaturase (sdd17m) gene in the Ổ3 desaturase-defective mutant ST1358 [71] resulted in EPA content as high as 26.4% of total fatty acid or 1.8 g/L at 28 °C [72]. While wild M. alpina accumulates only a small amount of the n-3 eicosatetraenoic acid (EPA, 20:4n-3) at low temperature (below 20 °C), this ETA was successfully produced by molecular breeding [73]. Further, by overexpression of the heterologous sdd17m gene controlled by an SSA2 promoter showing high transcriptional activity, ETA productivity in a Δ5 desaturase-defective mutant S14 reached 24.9% of total fatty acid at 28 °C [61].

Gene targeting may also be a valuable strategy for development of M. alpina strains producing SCO containing rare PUFAs. DGLA-producing transformants were constructed by disruption of the Δ5 desaturase gene, which encodes a key enzyme catalyzing the bioconversion of DGLA to ARA, in the lig4 gene-disrupted strain of M. alpina 1S-4 [74]. The uracil auxotroph of the lig4 gene-disrupted strain was transformed for disruption of the Δ5 desaturase gene through double crossing-over HR, and the targeting efficiency was calculated as 50%. The ratio of DGLA to total fatty acid in this disruptant reached 40.1%; however, no ARA was detected (Fig. 2). Thus, DGLA oil can be produced without ARA contamination. Such disruptants are superior to defective mutants (e.g., M. alpina 1S-4 mutant S14 constructed by chemical mutagenesis) for practical production of DGLA. Using the same methodology, MA-producing disruptants were constructed by disruption of the Δ12 desaturase gene (unpublished data) [75]. These disruptants showed no defects in growth, spore germination, and fatty acid production, but exhibited higher MA composition (8.4% of the total fatty acid) than the MA-producing Δ12 desaturase-defective mutant JT-180 (4.5%), with no accumulation of n-6 and n-3 PUFAs. Further application of gene targeting in M. alpina strains should facilitate improved PUFA productivity and help elucidate the enzyme pathways of PUFA biosynthesis.

**Table 4** Polyunsaturated fatty acid (PUFA) production by mutants and transformants derived from M. alpina 1S-4.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Target gene&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Parent&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Method&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Productivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARA</td>
<td>Δ12</td>
<td>JT-180</td>
<td>OE</td>
<td>Higher production (2.0 g/L/7 days, 39% of total fatty acids) than the M. alpina 1S-4 wild-type (1.2 g/L/7 days, 21%)</td>
</tr>
<tr>
<td></td>
<td>malce1</td>
<td>1S-4</td>
<td>OE</td>
<td>Higher ARA production (0.76 g/L/6 days, 34%) than the wild-type (0.68 g/L/6 days, 28%)</td>
</tr>
<tr>
<td></td>
<td>glelo</td>
<td>1S-4</td>
<td>OE</td>
<td>Higher ARA production (3.6 g/L/10 days, 28%) than the wild-type (1.9 g/L/10 days, 19%)</td>
</tr>
<tr>
<td></td>
<td>Pav.A5</td>
<td>1S-4</td>
<td>OE</td>
<td>Higher ARA composition (39%) and lower DGLA composition in the transformant than the wild-type (19% and 4%, respectively)</td>
</tr>
<tr>
<td></td>
<td>Ost.A6</td>
<td>1S-4</td>
<td>OE</td>
<td>Higher ARA composition (37%) in the transformant than the wild-type (19%)</td>
</tr>
<tr>
<td></td>
<td>Tri.A12</td>
<td>1S-4</td>
<td>OE</td>
<td>Higher ARA composition (36%) in the transformant than the wild-type (19%)</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>Δ6-1</td>
<td>JT-180</td>
<td>Ri</td>
<td>2.76 g/L/6 days, 68% of total fatty acid</td>
</tr>
<tr>
<td>EPA</td>
<td>s3</td>
<td>1S-4</td>
<td>ST1358</td>
<td>0.68 g/L, 38.2% of total fatty acid</td>
</tr>
<tr>
<td></td>
<td>sdd17m</td>
<td>S14</td>
<td>OE</td>
<td>1.8 g/L, 26.4% of total fatty acid</td>
</tr>
<tr>
<td>ETA</td>
<td>sdd17m</td>
<td>S14</td>
<td>GT</td>
<td>Higher DGLA composition (40%) than the mutant S14 strain (27%), with no ARA accumulation versus 0.2% in the mutant S14</td>
</tr>
<tr>
<td>DGLA</td>
<td>Δ5</td>
<td>1S-4</td>
<td>GT</td>
<td>Higher MA composition (8.4%) than the mutant JT-180 (4.5%), with no n-6 and n-3 PUFAs</td>
</tr>
<tr>
<td>MA</td>
<td>Δ12</td>
<td>1S-4</td>
<td>GT</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The genes, except for Pav.A5, Ost.A6, Tri.A12, and sdd17m, were derived from M. alpina 1S-4. AX, AX desaturase gene; Pav.A5, Pavlova salina A5 desaturase; Ost.A6, Ostreococcus lucimarinus Δ6 desaturase; Tri.A12, Tribolium castaneum Δ12 desaturase; sdd17m, Saprolegnia diclina Δ17 desaturase.

<sup>b</sup> JT-180, Δ12 desaturase-defective mutant; ST1358, Δ3 desaturase-defective mutant; S14, Δ5 desaturase-defective mutant.

<sup>c</sup> OE, overexpression; Ri, RNAi; GT, targeted gene disruption (gene-targeting).

**Conclusions and future perspectives**

The present review summarizes studies on lipogenesis in M. alpina 1S-4, the development of efficient gene manipulation systems for this strain, and the utilization of various M. alpina 1S-4 mutants for the production of beneficial PUFAs, especially ARA. The M. alpina 1S-4 wild-type, derivative mutants, and transformants are potential sources of triacylglycerols containing various...
PUFAs, including n-1, n-3, n-4, n-6, n-7, and n-9 PUFAs. By selective breeding of *M. alpina* and its mutants, it is possible to regulate the flow of both endogenous and exogenous fatty acids, thereby modifying the fatty acid profile and enhancing the production of desired (i.e., beneficial) PUFAs. Recent studies on *M. alpina* and its mutants have focused on molecular engineering of genes involved in PUFA biosynthesis and yielded strains with improved PUFA productivity. The molecular breeding of mutants and transgenic strains may make it possible to produce desired PUFAs efficiently. However, more efficient expression systems for enzymes involved in lipid synthesis, PUFA synthesis, and lipid conversion, as well as improved gene-silencing and targeted gene-disruption systems are needed to facilitate the breeding of *M. alpina* strains for large-scale production of functional lipids with industrial applications.

**Conflict of interest**

The authors declare no conflict of interest.

**Compliance with Ethics Requirements**

This article does not contain any studies with human or animal subjects.

**Acknowledgements**

This work was supported in part by a grant of the project of Advanced Low Carbon Technology Research and Development Program (ALCA) of the Japan Science and Technology Agency.

**References**


[10] Certik M, Sakuradani E, Shimizu S. Desaturase-defective fungal mutants: it is possible to regulate the flow of both endogenous and exogenous fatty acids, thereby modifying the fatty acid profile and enhancing the production of desired (i.e., beneficial) PUFAs. Recent studies on *M. alpina* and its mutants have focused on molecular engineering of genes involved in PUFA biosynthesis and yielded strains with improved PUFA productivity. The molecular breeding of mutants and transgenic strains may make it possible to produce desired PUFAs efficiently. However, more efficient expression systems for enzymes involved in lipid synthesis, PUFA synthesis, and lipid conversion, as well as improved gene-silencing and targeted gene-disruption systems are needed to facilitate the breeding of *M. alpina* strains for large-scale production of functional lipids with industrial applications.

**Conflict of interest**

The authors declare no conflict of interest.

**Compliance with Ethics Requirements**

This article does not contain any studies with human or animal subjects.

**Acknowledgements**

This work was supported in part by a grant of the project of Advanced Low Carbon Technology Research and Development Program (ALCA) of the Japan Science and Technology Agency.

**References**


Mortierella alpina produces a valuable polyunsaturated fatty acid.


Wynn JP, Ratcliffe C. Evidence that the rate-limiting step for the biosynthesis of arachidonic acid in Mortierella alpina is at the level of the 18:3 to 20:3 elongase. Microbiology 2000;146:2325–31.
Sakayu Shimizu is an Emeritus Professor at Kyoto University. He was a Professor in the Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University from 1992 to 2009, and a Professor in the Department of Bioscience and Biotechnology, Faculty of Bioenvironmental Science, Kyoto Gakuen University from 2009 to 2016. He completed his doctorate on fermentation physiology and applied microbiology in 1973 at Kyoto University. He was awarded a prize of the Vitamin Society of Japan in 2002, a prize of the Japan Society for Bioscience, Biotechnology, and Agrochemistry in 2003, and an International Enzyme Engineering Award in 2009. He is now serving as Chairman of the Board of Directors of the Japan Bioindustry Association. He is one of the pioneers of Single Cell Oil development and arachidonic acid rich-oil fermentation. He is also widely regarded for enzyme engineering research and has established several industrial processes for chiral chemical synthesis using microbial enzymes.

Jun Ogawa is a Professor at the Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University since 2009. He studied fermentation physiology and applied microbiology and completed his doctorate in 1995 at Kyoto University. In 2004, he was awarded a prize for Encouragement of Young Scientists from the Japan Society for Bioscience, Biotechnology, and Agrochemistry. In 2015, he was awarded the “Oleoscience Award” by the Japan Oil Chemists’ Society. He is serving as a Director of the Japan Society for Bioscience, Biotechnology, and Agrochemistry and is Chair of the Biotechnology Division of the American Oil Chemists’ Society (AOCS). His current research interests are screening and development of novel microbial functions useful in life sciences, food sciences, environmental sciences, and green chemistry, especially, fermentation physiology relating to functional lipid production.