# Production of 8,11,14,17-*cis*-Eicosatetraenoic Acid by Δ5 Desaturase-Defective Mutants of an Arachidonic Acid-Producing Fungus, *Mortierella alpina*

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**ABSTRACT:** Δ5 Desaturase-defective mutants of an arachidonic acid-producing fungus, *Mortierella alpina* 1S-4, accumulate large amounts of 8,11,14,17-*cis*-eicosatetraenoic acid (20:4ω3) when grown with linseed oil. One of the mutants, the S14 strain, produced 1.65 mg of 20:4ω3 per mL of culture medium (corresponding to 66.0 mg/g dry mycelia and 11.6% of total cellular fatty acids) when grown in a medium containing 1% glucose, 1% yeast extract, and 4% linseed oil methyl ester at 28°C for 2 d, and then at 16°C for 7 d. In a bench-scale fermentation in a 5-L jar fermenter, 20:4ω3 production reached 1.60 g/L of culture medium on the eighth day (corresponding to 77.3 mg/g dry mycelia and 26.0% of total cellular fatty acids). The cellular lipids of the S14 strain comprised 75.8% triacylglycerol (TG), 6.7% diacylglycerol, and 13.3% phospholipids (PL). The percentage of 20:4ω3 was higher in PL than in TG, and highest in phosphatidylcholine (32.6%).
JAOCS 74, 455–459 (1997).

**KEY WORDS:** Δ5 Desaturase, eicosatetraenoic acid, *Mortierella alpina,* 20:4ω3.

8,11,14,17-Eicosatetraenoic acid (20:4ω3) is a $C_{20}$ polyunsaturated fatty acid (PUFA) that belongs to the n-3 group. It is a double bond-positional isomer of arachidonic acid (AA, or 20:4ω6) and the precursor of 5,8,11,14,17-eicosapentaenoic acid (EPA, or 20:5ω3) in the n-3 pathway (Fig. 1). A small amount of 20:4ω3 is widely distributed in organisms that have the n-3 pathway, but its physiological role is hardly known. However, 20:4ω3 seems to be interesting because it is converted to prostaglandins in ram seminal vesicles (1,2) and may act as a natural analog of AA or EPA.

One reason for ignorance regarding its physiological role may be its poor availability. It is difficult to obtain 20:4ω3 because, for example, in fish oil the 20:4ω3 content is low (<5% of total fatty acids), and there are large amounts of AA and EPA, which are difficult to separate from 20:4ω3. Our studies on EPA production involving AA-producing *Mortierella* species showed that some 20:4ω3 was formed (about 0.5 mg/mL of culture medium) as a by-product (3–6). In these studies, we found that the fungi desaturated n-6 fatty acids to the corresponding n-3 acids at a low growth temperature (4,5), that α-linolenic acid added to the culture medium was converted to n-3 $C_{20}$ PUFA (6), and that the combination of these two phenomena led to maximum EPA production (1.88 mg/mL of culture medium) (3).

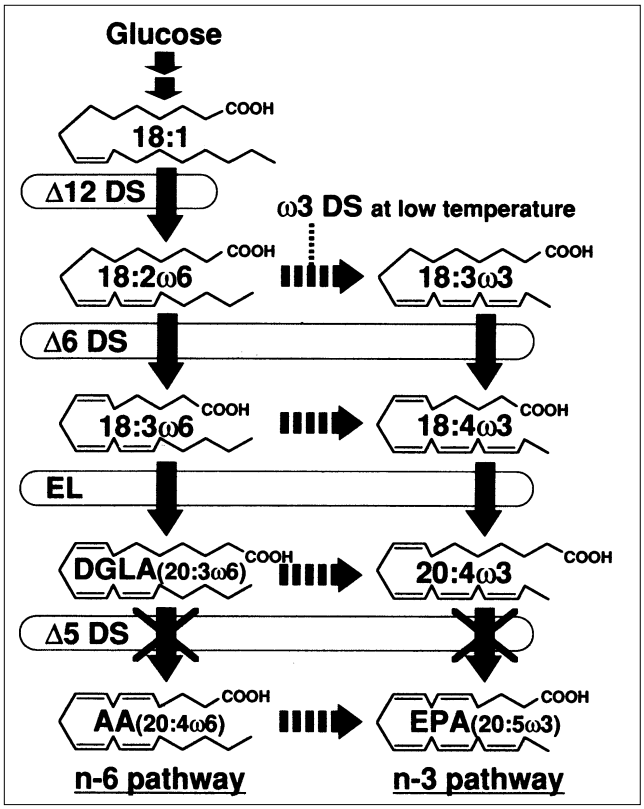


**FIG. 1.** Biosynthetic pathway for fatty acids in *Mortierella alpina* S14 and Mut44. Δ5 Desaturase is defective in the S14 and Mut44 strains. ω3 Desaturase is active at low temperature; DS, desaturase; EL, elongase; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid.

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We thought that 20:4ω3 accumulation by *Mortierella* in a manner similar to EPA production might be possible if Δ5 desaturation, i.e., conversion of 20:4ω3 to EPA, were effectively blocked (Fig. 1). As for Δ5 desaturase blocking, we developed two methods for dihomo-γ-linolenic acid (DGLA, or 20:3ω6) production by *Mortierella* fungi: the addition of a Δ5 desaturase inhibitor, such as sesamin (7,8) or curcumin (9), to the culture medium; and the use of Δ5 desaturase-defective mutants (10,11). The use of a Δ5 desaturase-defective mutant was found to boost 20:3ω6 productivity.

Here we examine 20:4ω3 production by using Δ5 desaturase-defective mutants *M. alpina* Mut44 (10,11) and S14 (12). The culture conditions for practical production of a single-cell oil with 20:4ω3 and the distribution of fatty acids in the major class of the resultant lipids are also described.

## MATERIALS AND METHODS

*Chemicals.* Linseed oil was purchased from Wako Pure Chemicals (Osaka, Japan). The fatty acid composition of the oil was as follows: palmitic (8.8%, by weight), stearic (4.8), oleic (11.8), linoleic (16.3), and α-linolenic (58.3) acids. Linseed oil methyl ester was prepared by transmethylation as described below. All other reagents were commercially available and as described previously (12).

*Microorganisms and cultivation. Mortierella alpina* Mut44 (10,11) and *M. alpina* S14 (12), which are Δ5 desaturase-defective mutants of *M. alpina* 1S-4, were inoculated as spore suspensions into 50-mL Erlenmeyer flasks that contained 10 mL of medium GY (1% glucose and 1% yeast extract, pH 6.0) supplemented with linseed oil methyl ester, and then incubated with reciprocal shaking at 120 rpm unless otherwise stated. For jar fermentation, the S14 strain was precultured at 28°C for 3 d in 100 mL of medium GY, and the resultant culture was inoculated into 1.8 L of medium GY supplemented with 4% linseed oil methyl ester and 0.01% Adekanol (Asahi Denka Industry, Tokyo, Japan) in a 5-L jar fermenter (Mitsuwa, Osaka, Japan). The cultivation periods and temperatures are indicated in the tables and figures.

*Fatty acid and lipid analyses.* Mycelia were harvested by suction filtration, washed with water and ether, and then dried at 100°C for 2 h for subsequent fatty acid analysis by gas–liquid chromatography (GLC) after transmethylation with methanolic HCl as described previously (13). Fungal lipids were extracted from wet mycelia with a chloroform–methanol–water system according to Folch *et al.* (14). The lipids were fractionated by thin-layer chromatography (13), and the fatty acid composition of each lipid fraction was analyzed by GLC as described above.

*Other methods.* Fungal growth was measured by determining the mycelial weight after drying at 100°C overnight. The glucose concentration of the culture medium was measured as described by Werner *et al.* (15). Mass spectra (MS) were recorded in the electron impact mode (70 eV) with a Hitachi M-80B apparatus (Tokyo, Japan) and proton nuclear magnetic resonance ($^1$H NMR) measurements were made with a Nicolet NT-360 apparatus (Madison, WI) with tetramethylsilane (TMS) as the internal standard.

## RESULTS

*Identification of 8,11,14,17-*cis*-eicosatetraenoic acid (20:4ω3).* 20:4ω3 Methyl ester (10 mg) was isolated from the lipids that were extracted from 25 g of wet mycelia of *M. alpina* S14 grown in medium GY with 2% linseed oil methyl ester at 20°C for 7 d, as described previously (13). MS of the isolated methyl ester showed peaks at *m/z* 318 ($M^+$; relative intensity, 1%), 222 (4), 175 (4), 161 (7), 147 (5), 135 (13), 119 (20), 108 (26), 93 (54), 79 (100), 69 (67), 55 (47), 41 (63), 29 (12), and 18 (9). The $^1$H NMR spectrum in $CDCl_3$, with TMS as an internal standard, showed signals at 0.97 (*t*, 3H, $CH_3$), 1.31 (*m*, 6H, $CH_2$), 1.61 (*m*, 2H, $CH_2$), 2.06 (*m*, 4H, $CH_2$), 2.32 (*t*, 2H, $CH_2$), 2.81 (*m*, 6H, $CH_2$), 3.67 (*s*, 3H, $CH_3$), and 5.37 ppm (*m*, 8H, C=C), indicative of four *cis*-double bonds interrupted by three methylene groups. The signal of the methyl end at 0.97 ppm indicates the existence of the n-3 structure. These data suggested that the isolated methyl ester was 8,11,14,17-*cis*-eicosatetraenoic acid methyl ester.

*Factors affecting 20:4ω3 production. Growth temperature.* The Δ5 desaturase-defective strains *M. alpina* Mut44 and S14 were cultivated in medium GY with 2% linseed oil at 28°C for 2 d, and then at 12–28°C for 7 d (Fig. 2). Both strains grew well at the tested temperatures. Production of 20:4ω3 by S14 was high at 16°C (0.96 mg/mL of culture medium) and 12°C (0.95 mg/mL), and decreased with increasing growth temperature above 16°C. The percentage of 20:4ω3 in the total cellular fatty acids was also high at 12°C (12.0%) and 16°C (11.9%) (Table 1). The production of DGLA, which is the n-6 fatty acid corresponding to 20:4ω3, was similarly high at low temperature. The other major fatty acids at 16°C in S14 were 18:3ω3 + 20:1 (31.1%), 18:1 (14.9%), 18:2ω6 (11.2%), and 16:0 (5.9%). EPA was not detected in S14. By contrast, some EPA was detected in Mut44, which in-

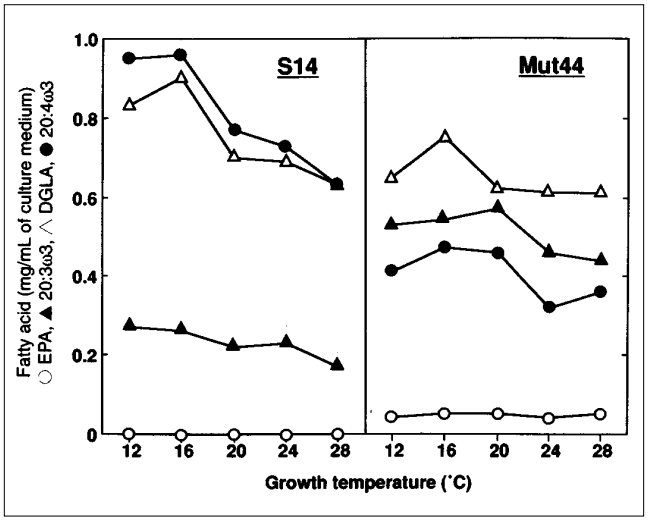


**FIG. 2.** Effect of growth temperature on fatty acid production. The fungi were cultivated in medium GY with 2% linseed oil methyl ester at 28°C for 2 d, and then at 12–28°C as indicated for 7 d. For Mut44, the amounts of 20:3ω3 shown in this figure include those of AA (less than 25%).

**TABLE 1**
**Fatty Acid Compositions of Mycelia of *Mortierella alpina* S14 and Mut44 Grown with Linseed Oil Methyl Ester[a]**

| Strain | Temp.[b] (°C) | Dry mycelial weight[c] (mg/mL of culture medium) | Fatty acid composition[c] (mol %) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 16:0 | 18:0 | 18:1 | 18:2 | 18:3ω6, 20:0 | 18:3ω3, 20:1 | 18:4ω3 | 20:2ω6 | DGLA | 20:3ω3, AA | 20:4ω3 | EPA | 24:0 |
| S14 | 12 | 17.3 | 6.5 | 5.2 | 20.6 | 15.4 | 2.1 | 20.0 | 1.7 | 0.3 | 10.5 | 3.5 | 12.0 | —[d] | 2.3 |
| | 16 | 15.6 | 5.9 | 4.2 | 14.9 | 11.2 | 2.1 | 31.1 | 1.5 | 0.3 | 11.1 | 3.2 | 11.9 | — | 2.5 |
| | 20 | 15.9 | 5.2 | 4.4 | 17.1 | 12.3 | 1.7 | 35.7 | 1.2 | 0.2 | 8.5 | 2.7 | 9.3 | — | 1.6 |
| | 24 | 15.8 | 6.3 | 3.8 | 17.4 | 12.2 | 2.1 | 32.8 | 1.7 | 0.4 | 8.6 | 2.9 | 9.2 | — | 2.8 |
| | 28 | 14.9 | 6.0 | 3.8 | 17.1 | 11.9 | 2.3 | 31.7 | 1.7 | 0.4 | 9.9 | 2.7 | 9.8 | — | 2.7 |
| Mut44 | 12 | 15.3 | 4.3 | 4.0 | 16.8 | 13.5 | 2.8 | 26.5 | 1.7 | 0.6 | 7.0 | 5.8 | 4.4 | 0.5 | 2.1 |
| | 16 | 15.2 | 4.5 | 4.1 | 15.2 | 12.5 | 3.4 | 33.5 | 1.9 | 0.6 | 8.8 | 6.3 | 5.5 | 0.6 | 3.1 |
| | 20 | 15.7 | 4.1 | 4.3 | 16.2 | 13.2 | 2.9 | 35.9 | 1.9 | 0.6 | 6.9 | 6.3 | 5.1 | 0.6 | 2.0 |
| | 24 | 14.5 | 5.7 | 3.7 | 14.5 | 12.2 | 3.9 | 30.3 | 1.9 | 0.8 | 9.0 | 6.8 | 4.7 | 0.7 | 6.0 |
| | 28 | 14.8 | 5.4 | 3.7 | 15.7 | 12.9 | 3.8 | 31.0 | 1.9 | 0.8 | 8.8 | 6.3 | 5.1 | 0.7 | 4.0 |

[a]The fungi were cultivated in medium GY containing 2% linseed oil methyl ester at 28°C for 2 d and then at 12–28°C for 7 d.
[b]The growth temperature was shifted to the indicated temperature from 28°C.
[c]Data are means of three independent determinations (SD is less than 8%). The first fatty acid of the paired ones is the major component.
[d]Undetectable.
Abbreviations: DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid.

dicated that the Δ5 desaturase in this strain is only partially defective and/or is produced at a low level, as reported previously (10–12). The production and percentage of 20:4ω3 in Mut44 were also high at 16°C (0.47 mg/mL and 5.5%, respectively), but lower than those of S14 at every tested temperature.

*Glucose concentration.* Strain S14 was cultivated in a medium that contained 1% yeast extract, 2% linseed oil methyl ester, and 0–3% glucose, pH 6.0, at 28°C for 2d, and then at 16°C for 7 d (Fig. 3A). 20:4ω3 Production was low (0.33 mg/mL of culture medium) when there was no glucose, increased to 0.90 mg/mL as the glucose concentration was increased to 1%, and remained almost constant (0.90–1.09 mg/mL) when the glucose concentration was 1–3%. In contrast, DGLA production and the dry cell weight drastically increased as the glucose concentration was increased. DGLA production was greater than 20:4ω3 production when the glucose concentration was above 1%, and reached 3.20 mg/mL when the glucose concentration was 3%.

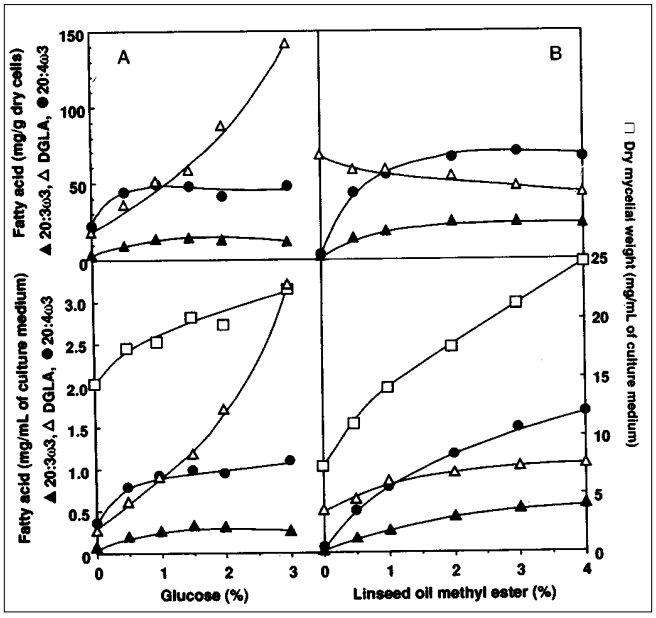


**FIG. 3.** Effects of glucose (A) and linseed oil methyl ester (B) concentrations on fatty acid production by strain S14. (A) Cultivated in a medium with 1% yeast extract, 2% linseed oil methyl ester, and 0–3% glucose as indicated at 28°C for 2 d, and then at 16°C for 7 d. (B) Cultivated in medium GY with 0–4% linseed oil methyl ester as indicated at 28°C for 2 d, and then at 16°C for 7 d.

*Linseed oil methyl ester concentration.* Strain S14 was also grown in medium GY with 0–4% linseed oil methyl ester (Fig. 3B). As linseed oil methyl ester was increased, 20:4ω3 production and the dry mycelial weight increased. Production of 20:4ω3 reached 1.65 mg/mL of culture medium (66.0 mg/g dry mycelia and 11.6% of total fatty acids) when the linseed oil methyl ester concentration was 4%. At 3%, the 20:4ω3 content was highest (69.6 mg/g dry mycelia). DGLA production was greater than 20:4ω3 production when the linseed oil methyl ester concentration was below 1%, and lower (0.94–1.06 mg/mL) at 2–4%.

*Bench-scale production.* Based on the culture conditions determined above, S14 was cultivated in a 5-L jar fermenter on 4% linseed oil methyl ester (Fig. 4). The mycelial mass yield became maximal on the sixth day and then remained unchanged (19.6–20.8 g/L) until the end of the cultivation. As the mycelial mass yield increased, 20:4ω3 production increased to 1.56 g/L of culture medium (70.0 mg/g dry mycelia and 23.0% of total fatty acids) on the sixth day and remained unchanged (1.55–1.61 g/L) until the end of the cultivation. On the twelfth day, 20:4ω3 production was 1.60 g/L of culture medium (77.3 mg/g dry mycelia and 26.0% of total fatty acids). DGLA production was much lower than 20:4ω3 production during the cultivation and reached 0.90 g/L on the ninth day. On the third day, the sum of the $C_{20}$ fatty acids was only 8.0%, and the fatty acid composition was similar to that of the linseed oil methyl ester supplement (Fig. 4B). The percentage of 20:4ω3 in the total fatty acids increased throughout the cultivation and reached 26.0% on the twelfth day, but that of DGLA was only 13.0%.

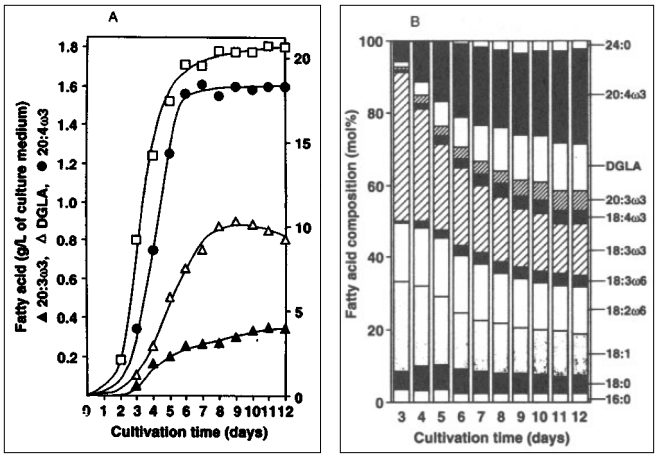


**FIG. 4.** 20:4ω3 Production in a jar fermenter. S14 was cultivated on 4% linseed oil methyl ester at 28°C for 2 d, and then at 18°C with aeration at 1 vol/vol/min and agitation at 300 rpm. Other conditions are given in the Materials and Method section. (A) Fatty acid production and cell growth. (B) Fatty acid composition during cultivation.

*Lipid analysis.* Extracted cellular lipids of S14 and Mut44 were separated into lipid classes (Table 2). About 75% (by mol) of the total lipids were comprised by triacylglycerol (TG) in both strains. As for S14, 77.2% of 20:4ω3 was distributed in TG. On comparison of the fatty acid compositions of the classes, 20:4ω3 was found to be abundant in phospholipids (PL), especially in phosphatidylcholine (PC) (32.6%) and phosphatidic acid (PA) (25.4%), whereas it was only 10.3% in TG. The percentage of 18:4ω3, which is the precursor of 20:4ω3, was also greater in PC (9.7%) and PA (7.9%) than in TG (1.9%). However, 18:3ω3 was more abundant in TG (34.9%) than in PL (3.8–9.0%). EPA was not detected in any class. The n-6 fatty acid profile was similar to that of the corresponding n-3 profiles; the percentages of DGLA and 18:3ω6 were greater in PL than in TG, whereas that of 18:2ω6 was smaller in PL.

In Mut44, 99.1% of 20:4ω3 was distributed in TG. The percentage of 20:4ω3 was greater in TG than in PL, which was quite different from the case of S14. EPA, rather than 20:4ω3, was abundant in phosphatidylethanolamine (PE) and PC. Most 18:4ω3 was distributed in PL, i.e., the percentages of 18:4ω3 in PC, PA, and PE were 26.2, 17.0, and 10.7%, respectively, while that in TG was only 2.0%. Such an extreme distribution also was observed for 18:3ω6, the n-6 fatty acid corresponding to 18:4ω3; i.e., its percentages in PC (28.2%), PA (22.7%), and PE (16.5%) were much greater than in TG (2.7%).

## DISCUSSION

We have reported that a small amont of 20:4ω3 was accumulated as a by-product in AA-producing *Mortierella* fungi when they were cultivated for EPA production (3–6). Here, by use of two Δ5 desaturase-defective mutants S14 (12) and Mut44 (10,11), which are defective in desaturation of 20:4ω3 to EPA, we developed a practical method for 20:4ω3 production.

There are two biosynthetic pathways for 20:4ω3 in S14 and Mut44 (Fig. 1). One is the n-3 pathway, in which exogenous 18:3ω3 is converted to 20:4ω3 by Δ6 desaturase and elongase. The other is ω3 desaturation from n-6 to n-3 fatty acids, which operates only at low temperature (4,5). In either case, the formed 20:4ω3 is not desaturated to EPA, or only slightly, and thus accumulates in mycelia of S14 and Mut44. Both strains produce a large amount of 20:4ω3 when grown at 16°C after preculturing at 28°C (Fig. 2). At a lower temperature, 20:4ω3 production is low, likely because the general metabolism—e.g., growth, fatty acid synthesis, energy supply for desaturation, etc.—may be slow, even though ω3 desaturation was active. At a higher temperature, 20:4ω3 pro-

**TABLE 2**
**Fatty Acid Compositions of Major Lipids of *Mortierella alpina* S14 and Mut44 Grown with Linseed Oil Methyl Ester[a]**

| Strain | Fraction[b] | Lipid composition (mol %) | Fatty acid composition[c] (mol %) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 16:0 | 18:0 | 18:1 | 18:2 | 18:3ω6, 20:0 | 18:3ω3, 20:1 | 18:4ω3 | 20:2ω6 | DGLA | 20:3ω3, AA | 20:4ω3 | EPA | 24:0 |
| S14 | TG | 75.8 | 9.7 | 3.5 | 15.1 | 10.6 | 2.2 | 34.9 | 1.9 | 0.1 | 7.1 | 3.0 | 10.3 | —[d] | 1.8 |
| | FA | 4.2 | 14.9 | 8.2 | 30.3 | 9.8 | 2.4 | 26.6 | 1.1 | — | 2.9 | — | 3.8 | — | — |
| | DG | 6.7 | 10.6 | 6.6 | 23.7 | 12.3 | 1.4 | 37.7 | 1.1 | — | 3.0 | 1.1 | 2.4 | — | — |
| | PE | 2.9 | 15.9 | 3.0 | 19.6 | 2.7 | 9.8 | 8.0 | 5.4 | — | 13.3 | 5.3 | 17.0 | — | — |
| | PC | 5.7 | 15.0 | 1.1 | 6.1 | 1.3 | 4.4 | 5.7 | 9.7 | — | 19.7 | 3.7 | 32.6 | — | 0.6 |
| | PS | 2.2 | 29.0 | 4.1 | 30.0 | 3.1 | 5.8 | 3.8 | — | — | 10.5 | 3.3 | 10.5 | — | — |
| | PA | 2.5 | 15.9 | 1.5 | 15.0 | 1.8 | 5.9 | 7.2 | 7.9 | — | 15.4 | 4.0 | 25.4 | — | — |
| Mut44 | TG | 75.5 | 8.0 | 3.1 | 14.2 | 11.9 | 2.7 | 42.3 | 2.0 | 0.3 | 4.1 | 5.7 | 3.8 | 1.0 | 0.9 |
| | FA | 3.9 | 13.2 | 9.0 | 23.9 | 9.8 | 2.1 | 32.2 | 1.2 | — | 2.9 | 4.4 | 1.3 | — | — |
| | DG | 9.8 | 7.2 | 3.0 | 21.3 | 15.2 | 2.7 | 47.0 | 1.4 | 0.7 | 0.5 | 1.5 | 0.2 | — | — |
| | PE | 3.5 | 11.5 | 2.2 | 13.3 | 7.8 | 16.5 | 15.7 | 10.7 | — | 1.1 | 16.3 | 1.9 | 2.3 | — |
| | PC | 3.9 | 11.4 | 1.4 | 4.2 | 5.3 | 28.2 | 12.8 | 26.2 | — | 0.4 | 7.0 | 1.5 | 1.8 | — |
| | PS | 1.6 | 28.6 | 2.6 | 15.3 | 14.0 | 9.9 | 15.7 | 3.7 | — | 2.7 | 7.5 | — | — | — |
| | PA | 1.8 | 15.9 | 3.2 | 8.9 | 7.8 | 22.7 | 15.2 | 17.0 | — | — | 9.3 | — | — | — |

[a]The fungi were cultivated in medium GY containing 2% linseed oil methyl ester at 28°C for 2 d and then at 12–28°C for 7 d.
[b]The growth temperature was shifted to the indicated temperature from 28°C.
[c]Data are means of three independent determinations (SD is less than 8%). The first fatty acid of the paired ones is the major component.
[d]Undetectable. For abbreviations see Table 1.

duction decreases, likely due to decreasing activities of ω3 and other desaturases. Of the two strains, S14 was excellent as a 20:4ω3 producer, because 20:4ω3 production was higher and EPA was undetectable in the mycelia. This difference is thought to be mainly due to the partially (Mut44) and almost completely (S14) defective Δ5 desaturase activities in the two strains.

Figure 3 shows that the productions of 20:4ω3 and of DGLA were competitive and varied in accordance with the ratio of linseed oil methyl ester and glucose. This behavior is reasonable because 18:3ω3, which constitutes about 60% of the total fatty acids in linseed oil, is only converted to n-3 fatty acids, i.e., not to n-6, whereas glucose is converted to n-6 fatty acids at first and then some of the latter are desaturated to n-3. It is also suggested that ω3 desaturation is much slower than the conversion of 18:2ω6 to DGLA because increasing glucose concentrations resulted in a large increase in DGLA production.

With optimization of the culture conditions, 20:4ω3 production reached 1.65 mg/mL of culture medium (66.6 mg/g dry mycelia and 11.6% of total fatty acids) in GY medium with 4% linseed oil methyl ester. Subsequently, we attempted 20:4ω3 production with strain S14 in a 5-L jar fermenter (Fig. 4). Production of 20:4ω3 was about 1.6 g/L of culture medium on the sixth day and then remained essentially unchanged until the end of the cultivation (12 d). On one hand, this value was almost the same as that obtained on a flask-scale. On the other hand, the much higher percentage of 20:4ω3 (26.0%) and its higher content (77.3 mg/g dry mycelia) were suitable for practical production. We thus showed that excellent productivity of 20:4ω3 can be achieved regardless of the cultivation scale.

Lipid analysis reveals that the main lipid class is TG in both S14 and Mut44 (Table 2). Among the n-3 fatty acids in S14, the percentages of 20:4ω3 and 18:4ω3 are higher in PL than in TG, and the amount of 18:3ω3 is low in PL, which suggests that the fatty acids in PL are selectively incorporated. Considering together that the percentages of DGLA and 18:3ω6 are also higher in PL, S14 seems to adapt to a low temperature (16°C) by selectively incorporating highly desaturated fatty acids into PL. This is also the case for Mut44. In Mut44, the most highly desaturated fatty acid, EPA, is well incorporated into PL. However, it is unclear why most 18:4ω3 and 18:3ω6 in Mut44 are distributed in PL. Some of the fatty acid metabolism in S14 and Mut44, other than the degree of the Δ5 desaturase defect, may be different.

## ACKNOWLEDGMENT

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