Biosynthesis of triacylglycerol in the filamentous fungus *Mucor circinelloides*

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Lipid metabolism was studied in 2-d-old liquid cultures of *Mucor circinelloides* grown at 25 °C. Under these conditions, oil accumulated to 0.5 g l⁻¹ with a γ-linolenic acid content (γ18:3) of 60 mg l⁻¹. The major labelled lipids in cultures incubated with [¹⁴C]acetate were triacylglycerol (TAG), phosphatidylcholine (PC) and phosphatidylethanolamine (PE). The proportion of label declined in the phospholipids and increased in TAG with time. [¹⁴C]18:1 and [¹⁴C]18:2 rapidly appeared in PC and PE and later accumulated in [¹⁴C]γ18:3. TAG-synthesizing capacity was greatest in the microsomal membrane fraction, which accumulated high levels of phosphatidic acid in the presence of glycerol-3-phosphate and acyl-CoA substrates at pH 7.0. Further metabolism of phosphatidic acid to diacylglycerol and TAG was achieved by increasing the pH to 8.0. Lysophosphatidic acid:acyl-CoA acyltransferase (LPAAT) activity was particularly high and may have accounted for the rapid accumulation of phosphatidic acid in the membranes. The glycerol-3-phosphate:acyl-CoA acyltransferase (GPAAT) and LPAAT were non-specific for a range of saturated and unsaturated species of acyl-CoA although the GPAAT showed a marked selectivity for palmitoyl-CoA and the LPAAT for oleoyl- and linoleoyl-CoA. γ-Linolenic acid was detected at all three positions of sn-TAG and was particularly enriched at the sn-3 position. The preparation of active in vitro systems (microsomal membranes) capable of the complete biosynthetic pathway for TAG assembly may be valuable in understanding the assembly of oils in future transgenic applications.

**Keywords**: *Mucor circinelloides*, gamma-linolenic acid, microsomes, triacylglycerol biosynthesis

INTRODUCTION

The biosynthesis of polyunsaturated fatty acids in plants (Stymne & Stobart, 1987; Harwood, 1988; Slabas et al., 1993) and animals (Brenner, 1982; Sprecher et al., 1995) and their assembly to produce storage fats and oils have been extensively studied. By comparison, and with the exception of oleaginous yeasts (Johnson et al., 1994; Sancholle & Lösel, 1995; Meesters & Eggink, 1996), few detailed biochemical studies on lipid synthesis in fungi have been undertaken. Of particular commercial interest is the production of so-called ‘speciality oils’ by microbes as alternative sources to those derived from oilseeds (Ratledge, 1993). In particular, there is considerable interest in the biosynthesis of γ-linolenic acid (C18:3, Δ6,9,12; γ18:3). This fatty acid is an important precursor in the production of prostaglandins in the body (Horrobin, 1990; Zurier, 1993), and supplementing dietary intakes with this component (usually oil extracted from evening primrose seeds) may have significant health benefits for a range of disorders (Horrobin, 1990, 1994). A number of fungi including *Mucor* and *Mortierella* species have also been identified which contain high levels of γ18:3 (Burnanova et al., 1990; Nakahara et al., 1992; Certik et al., 1993) and have been grown on an industrial scale (Suzuki, 1988).

Triacylglycerols (TAGs) are synthesized by the two-step acylation of sn-glycerol 3-phosphate to produce phosphatidic acid (PA), catalysed by the enzymes glycerol-3-phosphate:acyl-CoA acyltransferase (GPAAT) and...
lysophosphatidic acid:acyl-CoA acyltransferase (LP-AAT) (Stymne & Stobart, 1987). These enzymes usually display selectivity for the species of acyl-CoA used and govern the non-random distribution of fatty acids in sn-TAG (Griffiths et al., 1985). The PA formed is subsequently dephosphorylated to diacylglycerol (DAG) by phosphatidate phosphohydrolase. The DAG then serves as a precursor for TAG and this third acylation step is catalysed by diacylglycerol:acyl-CoA acyltransferase. In oilseeds, phosphatidylcholine (PC) has also been identified as an intermediate in oil biosynthesis and identified as an intermediate in oil biosynthesis and identified as an intermediate in oil biosynthesis and identified as an intermediate in oil biosynthesis and identified as an intermediate in oil biosynthesis and identified as an intermediate in oil biosynthesis and identified as an intermediate in oil biosynthesis and identified as an intermediate in oil biosynthesis and identified as an intermediate in oil biosynthesis and identified as an intermediate in oil biosynthesis and identified as an intermediate in oil biosynthesis and identified as an intermediate in oil biosynthesis and identified as an intermediate in oil biosynthesis and identified as an intermediate in oil biosynthesis. In oilseeds, the ability of fungal microsomal membranes to synthesize polyunsaturated TAGs is less certain. Holdsworth & Ratledge (1991), using the oleaginous yeast *M. circinelloides* (Jackson et al., 1998). In oilseeds, all of the enzymes required to catalyse TAG formation and to synthesize polyunsaturated fatty acids reside in the microsomal membrane fraction, which is derived largely from the endoplasmic reticulum (Stobart & Smyne, 1990). The homogenate was filtered through a glass sinter, diluted with fresh grinding buffer and centrifuged (20000 g, 15 min). The supernatant was filtered through a layer of Miracloth and centrifuged (100000 g, 60 min). The resultant pellet (microsomal membranes) was washed with cold 0·1 M potassium phosphate buffer, pH 7·2, and resuspended in a Potter-Elvehjem homogenizer using a small volume of the same buffer containing glycerol (20%, v/v). Microsomes were either used immediately or frozen rapidly at –80 °C, at which temperature they could be stored until required without significant loss of activity.

Oil bodies were obtained from the first 20000 g x 15 min centrifugation step and the fat pad was washed in fresh homogenization buffer and re-centrifuged at 20000 g for 15 min. Freshly prepared oil bodies were used in the experiments reported.

**Analytical procedures.** Lipids were extracted from whole cells by a modification of the method described by Bligh & Dyer (1959). The fungal cells were acidified with 0·15 M acetic acid (1 ml) followed by the addition of chloroform/methanol (1:2, v/v); 7·5 ml), distilled water (3·25 ml) and chloroform (2·25 ml). Phase separation was achieved by low-speed centrifugation and the lower chloroform phase containing the lipids was removed and evaporated under N₂. Samples were resuspended in a small volume of chloroform and stored at –20 °C until required. Membrane lipid extracts were made using chloroform/methanol/acetic acid (50:50:1, by vol.) and the lower chloroform phase was again recovered by low-speed centrifugation. Lipids were purified by TLC on pre-coated silica gel plates (Merck, silica gel 60). Neutral lipids were separated using hexane/diethyl ether/acetone (70:30:1, by vol.) and polar lipids using chloroform/methanol/acetic acid/water (170:30:20:7, by vol.) (Stobart & Smyne, 1990).

Lipids were located by lightly staining with iodine vapour and then removed from the plates for radioassay or transesterification for the analysis of fatty acids. The fatty acid methyl esters were prepared in 2·5% (v/v) sulphuric acid in anhydrous methanol (2 ml) (Griffiths et al., 1997) and quantified by GLC using heptadecanoic acid as internal standard on a GP 10% sp-2330 100/120 Chromosorb W AW (Supelco) column. Radio-labelled monoenoic, dienoic and trienoic fatty acid methyl esters were separated by argentation-TLC (Stobart & Smyne, 1990).

**Positional analysis of fatty acids in PA and TAG.** Phosphatidic acid was purified by TLC and eluted from the gel in methanol/chloroform (2:1, by vol.). The eluate was evaporated to dryness under N₂ and the residue redissolved in diethyl ether (1 ml). The lipid sample in diethyl ether was sonicated with 0·1 M sodium borate buffer, pH 8·9, and phospholipase A₁ (30 units) was added. After incubation, with vigorous mixing, at 25 °C for 80 min the fatty acids and lysophosphatidic acid were extracted in water-saturated butanol (Griffiths et al., 1985).

Stereochemical analyses of TAG were performed essentially as described by Christie (1982). TAGs purified by TLC were hydrolysed with Grignard reagent (ethyl magnesium bromide)
and the sn-1,2 diacylglycerols recovered were chemically converted to PCs. These were digested with phospholipase A₂; the fatty acids liberated represent those at the sn-2 position of the original TAG while those in lysophosphatidylcholine represent fatty acids at the sn-1 position. The fatty acids at the sn-3 position were calculated by subtraction using [position sn-3] = 3 × [TAG] – [position sn-1] – [position sn-2].

**Enzyme assays.** The specificity of GPAAT and LPAAT was determined using single-substrate acyl-CoA species as follows. Microsomes (equivalent to 0.5 mg protein) were incubated with [14C]palmitoyl-CoA, [14C]stearoyl-CoA, [14C]oleoyl-CoA or [14C]linoleoyl-CoA in the presence of glycerol 3-phosphate (400 nmol) in 0.1 M potassium phosphate buffer, pH 7.2, for 30 min at 25 °C. In studies on the acyl-CoA selectivity of these enzymes a mixture of 50 nmol of each of the acyl-CoA substrates was used.

LPAAT activity was also measured in a continuous assay using DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] and following the change in A₄₅₀ at 25 °C. The assay, modified from the method used by Styme & Stobart (1984) for lysophosphatidylcholine:acyl-CoA acyltransferase, routinely contained DTNB (50 nmol), oleoyl-CoA (20 nmol) and potassium phosphate buffer (50 mM), pH 7.2. Palmitoyl lysophosphatidic acid (30 nmol) was added to the sample cuvette and reactions were initiated by addition of microsomes (final vol. 1 ml). Other acyl-CoA donors were also examined. Initial rates were used to calculate activity using a molar absorption coefficient of 13.6 mM⁻¹ cm⁻¹.

**Protein determinations.** The bicinchoninic acid protein assay (Pierce and Warriner) standard protocol was used, with bovine serum albumin as a standard.

**RESULTS**

**Incorporation of [14C]acetate into lipids**

Acetate serves as a good substrate for the de novo biosynthesis of fatty acids and is readily taken up by tissues. The principal products of fatty acid synthesis are 16:0 and 18:1, so incorporation of acetate into these fatty acids would provide information on how these products were further used in desaturation and oil assembly. Two-day-old liquid cultures of *M. circinelloides* were incubated with [1-14C]acetate in time-course studies (up to 6 h), and the lipids were extracted, purified and the distribution of label in the various acyl constituents of the complex lipids determined.

The major labelled lipids from [14C]acetate were TAG, PC and to a lesser extent PE (data not given). The proportion of label in the phospholipids declined from initial values and there was a concomitant increase in radioactivity in TAG (data not given). The 14C-labelled fatty acid profiles of PC, PE and TAG over the time course are shown in Fig. 1. [14C]18:1 and [14C]18:2 rapidly appeared in PC and then declined with time; radioactivity accumulated in [14C]18:3 throughout the incubation. A similar trend was observed for PE, although a lower relative labelling of 18:2 and 18:3 occurred. Negligible levels of radioactivity were detected in phosphatidylinositol (PI) (data not shown). In TAG, [14C]18:1 was the most abundant fatty acid throughout the time course together with saturated fatty acids (16:0 plus 18:0). Labelling of the polyunsaturated fatty acids, 18:2 and 18:3, on the other hand, was low (approx. 25% after 6 h). Further characterization of oil assembly was undertaken following subcellular fractionation of the cells by measuring the in vitro activity of the so-called Kennedy pathway enzymes.
Incorporation of \(^{14}\text{C}\)glycerol 3-phosphate into subcellular fractions

Oil-body and microsomal membrane fractions were assayed for TAG biosynthetic activity using \(^{14}\text{C}\)-glycerol 3-phosphate and oleoyl-CoA as substrates. Most of the activity (90\%) was recovered in the microsomes (equivalent to 2.3 \(\mu\)mol \(^{14}\text{C}\)glycerol 3-phosphate incorporated into total lipids from 12 g wet weight of mycelia) and the remaining 10\% was detected in the oil-bodies. PA was the predominantly labelled lipid in both preparations (78\%), with only small amounts (approx. 5\%) in each of PC, PE, DAG and TAG. The results indicated that acylation of glycerol 3-phosphate to PA had occurred and that further metabolism of PA to DAG by the phosphohydrolase enzyme was slow, with concomitantly little increase in label in TAG. We have shown that the activity of phosphohydrolase in oilseed microsomes can be manipulated by the availability of Mg\(^{2+}\) (Griffiths et al., 1985). Addition of Mg\(^{2+}\) (10 mM) and EDTA (10 mM) alone or in combination failed to stimulate the further metabolism of PA to DAG and TAG (data not shown). However, increasing the pH of the reaction mixture from 7.0 to 8.0 resulted in a significant increase in label in PC (almost fourfold), with radioactivity slowly accumulating in both DAG and TAG (Table 1).

Spectrophotometric determination of LPAAT activity

The spectrophotometric determination of LPAAT activity was performed using single-substrate \(^{14}\text{C}\)acyl-CoA in the presence of unlabelled glycerol 3-phosphate. When acyl-CoA substrates are presented to microsomal membranes they can, potentially, enter into an acyl-exchange with acyl groups, predominantly the C5, polyunsaturated fatty acids, at position sn-2 of PC (Stymne & Stobart, 1984; Griffiths et al., 1985). This results in unlabelled fatty acids being made available to the acyl-CoA pool for utilization in the acylation of glycerol 3-phosphate in addition to the exogenously supplied radiolabelled acyl-CoAs. The results showed that 16:0 and 18:1 were most effectively incorporated into PA (Table 2). 18:2 was also efficiently utilized whereas 18:0, on the other hand, was a relatively poor substrate. Stereo-specific analysis (Table 2)

**Table 1.** Incorporation of \(^{14}\text{C}\)glycerol 3-phosphate into lipids by microsomal membranes

Microsomal membranes (equivalent to 0.5 mg protein) were incubated with \(^{14}\text{C}\)glycerol 3-phosphate (500 nmol; specific activity 2559 d.p.m. nmol\(^{-1}\)) and oleoyl-CoA (100 nmol) in phosphate buffer, pH 8.0 (0.1 M, 1 ml final volume); the lipids were extracted at the times indicated and the radioactive distribution determined. Results are representative of an experiment repeated twice.

| Time (min) | Radioactivity (%) | Total [nmol (mg protein)]
|---|---|---
| | PA | PC | PE | DAG | TAG |
| 10 | 72 | 18 | 2 | 5 | 4 | 110 |
| 20 | 61 | 23 | 2 | 6 | 8 | 130 |
| 60 | 50 | 25 | 2 | 12 | 11 | 144 |
| 100 | 44 | 26 | 2 | 14 | 14 | 140 |

**Table 2.** Incorporation of \(^{14}\text{C}\)acyl-CoAs into microsomal phosphatidate

Microsomes (0.5 mg protein) were incubated with 200 nmol of either \(^{14}\text{C}\)16:0-CoA, \(^{14}\text{C}\)18:0-CoA, \(^{14}\text{C}\)18:1-CoA or \(^{14}\text{C}\)18:2-CoA (single substrate) or with 50 nmol of each substrate (mixed substrate) in the presence of glycerol 3-phosphate (400 nmol) in phosphate buffer, pH 7.2, for 30 min. Lipids were extracted and the positional distribution of radioactivity in phosphatidate was determined. Similar results were obtained on two other occasions (see typically <5\% of the mean for each value). ND, Not detected.

| 14C-labelled fatty acid | Positional distribution (nmol)*
|---|---
| | sn-1 | sn-2 |
| Single substrate | | | |
| 16:0 | 26.2 (54) | 22.3 (46) |
| 18:0 | 5.6 (68) | 2.7 (32) |
| 18:1 | 15.4 (34) | 29.8 (66) |
| 18:2 | 6.4 (33) | 13.1 (67) |
| Mixed substrate | | | |
| 16:0 | 7.1 (87) | 1.1 (13) |
| 18:0 | ND | ND |
| 18:1 | 2.1 (38) | 3.3 (62) |
| 18:2 | 2.4 (31) | 5.5 (69) |

* The numbers in parentheses represent the percentage distribution between the sn-1 and sn-2 positions.

**Utilization of \(^{14}\text{C}\)acyl-CoA substrates in the acylation of glycerol 3-phosphate**

The accumulation of radioactivity from \(^{14}\text{C}\)glycerol 3-phosphate into PA at pH 7 provided an opportunity for studying the acyl specificity and selectivity of the acyltransferases for different acyl-CoA substrates. The specificity of the acylating enzymes was investigated using single-substrate \(^{14}\text{C}\)acyl-CoA in the presence of unlabelled glycerol 3-phosphate. When acyl-CoA substrates are presented to microsomal membranes they can, potentially, enter into an acyl-exchange with acyl groups, predominantly the C5, polyunsaturated fatty acids, at position sn-2 of PC (Stymne & Stobart, 1984; Griffiths et al., 1985). This results in unlabelled fatty acids being made available to the acyl-CoA pool for utilization in the acylation of glycerol 3-phosphate in addition to the exogenously supplied radiolabelled acyl-CoAs. The results showed that 16:0 and 18:1 were most effectively incorporated into PA (Table 2). 18:2 was also efficiently utilized whereas 18:0, on the other hand, was a relatively poor substrate. Stereospecific analysis (Table 2)
Table 3. Positional distribution of fatty acids in TAG

Purified TAG was subjected to random chemical deacylation followed by derivatization of purified sn-1,2 DAG to PC. The distribution of fatty acids at the sn-1 and sn-2 positions was then determined following phospholipase A2 digestion and GLC analysis of the fatty acids, and position sn-3 determined by calculation. Comp, relative acyl composition of each position; Dist, relative distribution of each acyl group between the three positions; ND, not detected.

<table>
<thead>
<tr>
<th>Position</th>
<th>Fatty acid composition/distribution (mol %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16:0</td>
</tr>
<tr>
<td>sn-1</td>
<td>61.4</td>
</tr>
<tr>
<td>sn-2</td>
<td>8.9</td>
</tr>
<tr>
<td>sn-3</td>
<td>48.2</td>
</tr>
<tr>
<td>Total*</td>
<td>39.0</td>
</tr>
</tbody>
</table>

* Actual composition of the triacylglycerol molecule determined by directly methylating the purified lipid.

2) showed that 16:0 was almost evenly distributed between the sn-1 and sn-2 position whereas 18:1 and 18:2 were preferentially acylated to the sn-2 position. 18:0, however, was preferentially located at the sn-1 position.

To investigate the acyl selectivity of the acyltransferases, microsomes were incubated with mixed \[^{14}C\]acyl-CoA substrates and glycerol 3-phosphate. Under these conditions 16:0 was extensively used for the acylation of position sn-1 of glycercyl 3-phosphate whereas both \[^{14}C\]18:1 and \[^{14}C\]18:2 were preferentially acylated to the sn-2 position and \[^{14}C\]18:0 was not utilized (Table 2).

Positional analysis of TAG

Purified TAG was subjected to random chemical deacylation followed by derivatization of purified sn-1,2 DAG to PC. The distribution of fatty acids at the sn-1 and sn-2 positions was then determined following phospholipase A2 digestion and GLC analysis of the fatty acids. The acyl composition of sn-3 was determined indirectly by calculation (see Methods). 16:0, a major fatty acid in the fungus, was present largely in positions sn-1 and sn-3 with only a small proportion located at the sn-2 position (Table 3). Only low levels of 18:0 were present in TAG (4% total) and were evenly distributed between positions sn-1 and sn-2. 18:1 was found to reside in similar amounts in position sn-1 and sn-3, with the highest abundance in the sn-2 position. 18:2 was largely located in position sn-2, with approximately 25% present at sn-3 and little (approx. 8%) at sn-1. γ18:3 was largely present at position sn-3, with the lowest level at the sn-1 position.

DISCUSSION

TAG biosynthesis occurs in the endoplasmic reticulum in oilseeds (Stymne & Stobart, 1987), although some activity has been reported in oil-body preparations (Gurr et al., 1974). In subcellular fractionation studies with the oleaginous yeast Candida curvata, however, it was concluded that PA formation was mainly located in the mitochondrion and that recombining all subcellular fractions (lipid bodies, spheroplasts, mitochondria and soluble supernatant) was necessary to achieve TAG-synthesizing activity (Holdsworth & Ratledge, 1991). In the present study microsomal membranes contained the highest activity of TAG-synthesizing enzymes. The membranes were capable of the complete assembly of TAG from precursor acyl-CoA and glycerol 3-phosphate substrates at rates comparable to those reported for oilseeds (Stymne & Stobart, 1985; Griffiths & Harwood, 1991).

Labelling studies with \[^{14}C\]acetate indicated that fatty acids synthesized de novo were rapidly incorporated into the accumulating oil, with negligible quantities detected in the intermediates of the pathway, namely PA and DAG. The pattern of fatty acids synthesized indicates that Δ9, Δ12 and Δ6 desaturases were active and that labelling of the acyl moieties of the two phospholipids, PC and PE, occurred initially and with time radioactivity accumulated in TAG. The kinetics of labelling of the two phospholipids and the accumulation of \[^{14}C\]18:3 and the concomitant decrease in radioactivity in \[^{14}C\]18:1 indicate a role for these complex lipids in fatty acid desaturation. In oilseeds PC is the major substrate for Δ12 and Δ6 desaturase activity, with the activity of the latter enzyme restricted to linoleoyl groups esterified exclusively to the sn-2 position (Griffiths et al., 1988a). In microsomal membrane preparations incubated with NADH we have also previously observed increases in the mass of γ18:3 in endogenous PE (Stymne et al., 1987). However, studies in vitro, with microsomal membranes under conditions of rapid TAG biosynthesis (i.e. in the presence of acyl-CoA and glycerol 3-phosphate) have indicated that PE is
not a prime substrate in the production of polyunsaturated fatty acids for other lipids (Stymne & Stobart, 1987). This was further supported by results of studies in vivo using tissue slices of developing cotyledons in which PC was almost exclusively the only phospholipid labelled with acetate or a range of labelled fatty acid precursors (Griffiths et al., 1988b). In M. circinelloides, PI has been suggested as a major substrate for γ18:3 synthesis based on changes in the endogenous mass of fatty acids in microsomal membrane preparations (Kendrick & Ratledge, 1992). However, in the present study there was little labelling of PI from acetate under conditions in vivo. Recently, we have shown that PC is the major substrate for the production of 18:2 and also of γ18:3 in M. circinelloides using microsomal membranes supplied with acyl-CoA substrates under desaturating conditions (i.e. in the presence of NADH) (Jackson et al., 1998).

The phosphatidic acid phosphohydrolase (PAP) enzyme in M. circinelloides appears to be insensitive to changes in Mg²⁺ concentration, unlike the safflower enzyme (Griffiths et al., 1985). In mammalian tissues, PAP has been suggested to be the rate-limiting step in TAG assembly and is regulated by enzyme translocation between the cytosolic and endomembranes (Hopewell et al., 1985). A similar regulatory mechanism has been proposed for the oilseed enzyme (Ichiha et al., 1990). The purified enzyme from Saccharomyces was also reported to have an absolute requirement for Mg²⁺ (Hosaka & Yamashita, 1984). The accumulation of PA in membranes used in the present study suggests that it may also be a rate-limiting step in the production of TAG in M. circinelloides.

Accumulation of PA in the microsomal membranes, especially at the lower pH, allowed investigation of the acyl specificity and selectivity properties of glycerol 3-phosphate-acylating enzymes. Palmitoyl-CoA used as a single substrate was almost evenly distributed between position sn-1 and sn-2 of PA, indicating that the first and second acyltransferases (converting glycerol 3-phosphate to lysophosphatidate and to PA by acylation at the sn-2 position) were not specific for unsaturated fatty acids, unlike the oilseed enzyme (Griffiths et al., 1985). The unsaturated acyl-CoA species were also acylated to both positions of PA. Therefore neither GPAAT nor LPAAT showed specificity for certain acyl-CoA substrates, with saturated and unsaturated species being acylated to both positions. In selectivity studies, however, with mixed acyl-CoA being presented to membranes, GPAAT showed a greater selectivity for palmitate whereas LPAAT showed selectivity for unsaturated fatty acids. Studies on the rate of acylation of lysophosphatidic acid by acyl-CoA:1-acylglycerol 3-phosphate acyltransferase (LPAAT) indicated that the activity of this enzyme was particularly high compared with that from sunflower and guinea pig; this may account for the rapid accumulation of PA in M. circinelloides membranes. The distribution of fatty acids between the three positions of the glycerol backbone also reflects, to some extent at least, the selectivity properties in vivo of the glycerol-acylating enzymes. The analyses revealed a strong agreement between the distribution of acyl groups in TAG and the selectivity properties of the acylation enzymes. γ18:3 was present at all three positions and was particularly enriched at the sn-3 position, perhaps indicating some selectivity for this fatty acid by the diacylglycerol:acyl-CoA acyltransferase. The ability of microsomal membranes to carry out the complete assembly of TAGs may facilitate future work aimed at understanding the regulation of this process in fungi and help in the future improvement of microbial oil quality for biotechnical applications.

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