I. INTRODUCTION

The n-6 and n-3 polyunsaturated fatty acids (PUFAs, see Fig. 1) are essential in human nutrition. They are building blocks in the membrane lipid biogenesis and play important roles in the structure and function of the biological membranes. The dietary effects of these PUFAs have also attracted increasing interest because of their unique biological activities, such as lowering of plasma cholesterol level, prevention of thrombosis, etc. Some of them, i.e. dihomo-γ-linolenic acid (8,11,14-cis-eicosatrienoic acid, DGLA), arachidonic acid (5,8,11,14-cis-eicosatetraenoic acid, ARA) and 5,8,11,14,17-cis-eicosapentaenoic acid (EPA) are natural precursors of a large family of structurally related C-20 compounds, such as prostaglandins, leucotrienes etc., all of which are potent biological regulators. This all suggests that PUFAs are highly important substances in the pharmaceutical, medical and nutritional fields. The available sources relatively rich in these PUFAs have not been known except for some seed oils (γ-linolenic acid, 6,9,12-cis-octadecatrienoic acid, GLA) and fish oils (EPA and 4,7,10,13,16,19-cis-docosahexaenoic acid, DHA).

We found that several fungi produce large amounts of lipids rich in DGLA, ARA or EPA, or all of these C-20 PUFAs. They are new and promising sources of C-20 PUFAs. Here, we summarize our recent results [1-4] on microbial production of C-20 PUFAs.

II. ARACHIDONIC ACID

We assayed C-20 PUFA productivities in a variety of microorganisms. Most C-20 PUFA producers were found to be Mucorales and Entomophthorales fungi. Among them, 60 Mortierella strains and 45 isolates from natural sources produced large amounts of C-20 PUFAs of the n-6 family (i.e. ARA and DGLA) together with C-18 PUFAs of the same family (i.e. GLA). The mycelial ARA levels of these fungi accounted for more than 15% of the total extractable fatty acids. Through this screening, we selected two soil isolates which are taxonomically identified as Mortierella alpina as the most promising producers of ARA [1,5]. When cultured in 2000-liter fermentor, M. alpina 1S-4 produced 22.5 g/l of mycelia (dry weight) containing 44.0%, by weight, of lipids, ARA comprising 31.0% of the total fatty acids, after 10 days cultivation at 28°C (Fig. 2a). The ARA in the harvested mycelia could be further enriched when the mycelia were allowed to stand for a further few
days at room temperature. The ARA content of the resultant mycelia reached nearly 70% of the total fatty acids (Fig. 2b) [6]. The triglyceride containing ARA was obtained from the mycelia in a recovery of 80–90%, from which ARA could be isolated as the ethyl ester in a good recovery through a simple procedure including liquid-liquid partition chromatography.

III. DIHOMO-\(\gamma\)-LINOLENIC ACID

Most ARA-producing fungi accumulated small amounts of DGLA as a by-product in ARA production [1,7]. Because DGLA is converted to ARA through the n-6 route, the ARA accumulated in the mycelia must be produced via DGLA, suggesting that all the ARA-producing fungi potentially have the ability to produce large amounts of this fatty acid. In order to obtain a higher yield of DGLA, we attempted to repress the conversion of DGLA to ARA (i.e. \(\Delta^5\)-desaturation). Among various substances tested, sesame oil, when added to the culture medium, was found to cause a marked decrease in mycelial ARA level [8]. On addition of 3% sesame oil, the production of DGLA by M. alpina 1S-4 reached 1.7 g/l, whereas the production of ARA was only 0.7 g/l. These values were 3.4-fold higher and 5.0-fold lower than those for DGLA and ARA without sesame oil, respectively. This phenomenon was suggested to be due to a specific inhibition of the conversion of DGLA to ARA by the oil. The effective factors responsible for this phenomenon were isolated and identified as (+)-sesamin and related lignan compounds (Fig. 3) [4,9,10]. In a study on optimization of the culture conditions for the production of DGLA by M. alpina 1S-4, a medium containing glucose, yeast extract and the non-oil fraction of sesame oil was found to be suitable for the production. Under the optimal conditions in a 50-liter fermentor, the fungus produced 2.57 g/l of DGLA (99 mg/g dry mycelia) (Fig. 2c). This value accounted for 26.8% of the total mycelial fatty acids. The mycelia were also rich in ARA (55 mg/g dry mycelia, 14.9%) [8].
The results obtained in experiments with both a cell-free extract of *M. alpina* 1S-4 and a rat liver microsomes demonstrated that these lignans specifically inhibit Δ5-desaturase at low concentrations. On the other hand, Δ6-, Δ9- and Δ12-desaturases were not inhibited by the lignans. Kinetic analysis showed that sesamin is a noncompetitive inhibitor (Ki for rat liver Δ5-desaturase, 155 µM) [9]. Similarly, (-)-asarinein and (-)-epiasarinin, the stereoisomers of (+)-episesamin and (+)-sesamin, respectively, isolated from a Chinese medicine "Saishin" (Asiasari radix) also showed specific noncompetitive inhibition of Δ5 desaturase. The inhibitory effect on Δ5 desaturase of these lignans were in order of (+)-sesamin > (-)-epiasarinin > (-)-asarinein > (+)-episesamin. These studies are the first to demonstrate the occurrence of desaturase inhibitors in nature [9,11].

### IV. EICOSAPENTAENOIC ACID

We found that lowering the cultivation temperature caused the additional accumulation of a PUFA with five double bonds, EPA, by all the ARA producers tested [1,7,12]. The mycelial lipids extracted from *M. alpina* 1S-4 grown at 12°C contained 47.6% phospholipids and 35.7% triglycerides. More than 60% of the EPA accumulated in the mycelia was found in the phospholipid fraction. On the other hand, the lipids from the mycelia grown at 28°C contained only 21.6% phospholipids, the triglyceride fraction comprising 73.5% of the total extractable lipids. The results of experiments with cell-free extracts of the fungus demonstrated that the enzyme(s) that catalyzes the formation of EPA is produced even when it is grown at high temperature, but that the reaction(s) yielding EPA does not take place at high temperature and an n-6 PUFA, probably ARA, is the precursor of EPA [7]. The results suggest that an enzyme(s) or enzyme system catalyzing the methyl-end directed desaturation of ARA (Δ17-desaturation) is activated on cold adaptation. The resultant EPA may be necessary for maintaining the proper membrane fluidity in a low temperature environment.

Several ARA-producing Mortierella strains accumulated detectable amounts of EPA in their mycelia when grown in media containing α-linolenic acid (ALA) [13]. This observation suggests that the n-3 route occurs in these fungi as well as in animals, although they lack the ability to synthesize ALA. This route seems to be independent of the growth temperature, because EPA production takes place even on growth at 28°C. The ability of the Mortierella fungi to convert added ALA to EPA is very promising from a biotechnological viewpoint, because there are various kinds of easily available natural oils containing ALA, and it is expected that they can be converted to oils rich in EPA on incubation with these fungi. We examined the potential of such natural oils as precursors of EPA and found that linseed oil, in which ALA amounts to about 60% of the total fatty acids, is the most suitable for EPA production. Under the optimal culture conditions, *M. alpina* 2O-17 converted

### TABLE 1

Comparison of EPA productivities and mycelial fatty acid profiles of two Mortierella strains under different culture conditions

<table>
<thead>
<tr>
<th>Strain, conditions and responsible route for EPA production</th>
<th>Productivity</th>
<th>Mycelial fatty acid composition in weight %</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mycelial mass (g/l)</td>
<td>Mycelial EPA content (g/g dry mycelia)</td>
</tr>
<tr>
<td><em>M. alpina</em> 1S-4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12°C, n-6</td>
<td>11.1</td>
<td>27.0</td>
</tr>
<tr>
<td>28°C, linseed oil, n-3</td>
<td>24.1</td>
<td>41.0</td>
</tr>
<tr>
<td>12°C, linseed oil, n-3, n-6</td>
<td>28.2</td>
<td>66.6</td>
</tr>
<tr>
<td><em>M. alpina</em> 2O-17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12°C, n-6</td>
<td>17.0</td>
<td>29.0</td>
</tr>
<tr>
<td>28°C, linseed oil, n-3</td>
<td>32.4</td>
<td>41.5</td>
</tr>
</tbody>
</table>

*a* See [7,12-14] for details.
5.1% of the ALA in the added linseed oil into EPA, the EPA production reaching 1.35 g/l (41.5 mg/g dry mycelia). This value is 2.8-fold higher than that obtained under low temperature growth conditions. The resultant lipid is rich in either ARA or EPA. The EPA production was further stimulated when ARA-producing fungi were grown in a medium containing linseed oil at low temperature. This phenomenon was suggested to be mainly due to that the low temperature-dependent production of EPA from ARA formed through the n-6 route and the conversion of the ALA in the added linseed oil to EPA through the n-3 route took place at the same time [14]. Stimulation of the n-3 route itself at low temperature was also suggested to contribute to the increased EPA production. The amount of EPA accumulated reached 1.88 g/l (66.6 mg/g dry mycelia) on cultivation of M. alpina IS-4 with 3% linseed oil at 12°C. These results are summarized in Table 1.

REFERENCES


