Characterization of Mannosylerythritol Lipids Containing Hexadecatetraenoic Acid Produced from Cuttlefish Oil by *Pseudozyma churashimaensis* OK96

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Abstract: Biosurfactants are surface-active compounds produced by microorganisms. Mannosylerythritol lipids (MEL) are promising biosurfactants produced by Ustilaginomycetes, and their physicochemical and biochemical properties differ depending on the chemical structure of their hydrophilic and/or hydrophobic moieties. To further develop MEL derivatives and expand their potential applications, we focused our attention on the use of cuttlefish oil, which contains polyunsaturated fatty acids (e.g., docosahexaenoic acid, C₂₂:₆, and eicosapentaenoic acid, C₂₀:₅) as the sole carbon source. Among the microorganisms capable of producing MEL, only nine strains were able to produce them from cuttlefish oil. On gas chromatography-mass spectrometry (GC/MS) analysis, we observed that *Pseudozyma churashimaensis* OK96 was particularly suitable for the production of MEL-A, a MEL containing hexadecatetraenoic acid (C₁₆:₄) (23.6% of the total unsaturated fatty acids and 7.7% of the total fatty acids). The observed critical micelle concentration (CMC) and surface tension at CMC of the new MEL-A were $5.7 \times 10^{-6}$ M and 29.5 mN/m, respectively, while those of MEL-A produced from soybean oil were $2.7 \times 10^{-6}$ M and 27.7 mN/m, respectively. With polarized optical and confocal laser scanning microscopies, the self-assembling properties of MEL-A were found to be different from those of conventional MEL. Furthermore, based on the DPPH radical-scavenging assay, the anti-oxidative activity of MEL-A was found to be 2.1-fold higher than that of MEL-A produced from soybean oil. Thus, the newly identified MEL-A is attractive as a new functional material with excellent surface-active and antioxidative properties.

Key words: *Pseudozyma*, glycolipid, mannosylerythritol lipid, biosurfactant, cuttlefish oil

1 INTRODUCTION

Biosurfactants (BS) are surface-active compounds produced by various microorganisms. In recent years, BS have attracted considerable interest due to their unique properties such as biodegradability, mild production conditions, and multi-functionality¹.². Mannosylerythritol lipids (MEL), one of the most promising glycolipid BS, are constituted by 4-O-β-D-mannopyranosyl-meso-erythritol as the hydrophilic moiety, and fatty acid and/or acetyl groups as the hydrophobic moiety (Fig. 1). Not only MEL show excellent surface-active and self-assembling properties, but they also display versatile biochemical functions, including antitumor and cell differentiation activities with respect to human leukemia, rat pheochromocytoma, and mouse melanoma cells.³.⁴. We recently demonstrated that MEL-A, a MEL containing hexadecatetraenoic acid, shows ceramide-like skin care⁵ and hair care properties⁶, and thus it could potentially become a new cosmetic ingredient.

*Pseudozyma antarctica*, which is an anamorphic basidionymycetous yeast, is well known as a MEL producer(mainly MEL-A). *P. rugulosa*, *P. aphidis*, and *P. parantarctica* were also found to predominantly produce large amounts of MELs(mainly MEL-A)⁷, while *P. tsukubaensis* was able to form a diastereomer type of MEL-B⁸.⁹. Moreover, *P.
MEL-A: $R_1 = R_2 = Ac$
MEL-B: $R_1 = Ac$, $R_2 = H$
MEL-C: $R_1 = H$, $R_2 = Ac$
($n = 4 - 16$)

Fig. 1 Chemical Structure of Mannosylerythritol Lipids.

graminicola, P. hubeiensis, P. shanxiensis, and P. siamensis were found to be MEL-C producers\cite{10-13}. In addition, we recently isolated P. churashimaensis OK96 as a novel MEL-A producer\cite{14}.

While di-acylated MEL (i.e., MEL-A, MEL-B, and MEL-C) show excellent surface tension-lowering activities and low critical micelle concentration (CMC) regardless of the acetyl and fatty acid composition, their self-assembling properties are drastically dependent on those factors. In fact, MEL-A produced from soybean oil by P. antarctica T-34 self-assembles into various lyotropic liquid crystals such as sponge ($L_0$), inverted hexagonal ($H_2$), and lamella ($L_\alpha$) phases, while MEL-B show myelin and $L_\beta$ phases at a wide range of concentrations\cite{15, 16}. In addition, based on the water-penetration observation, the patterns of liquid crystalline phases of MEL-C differ depending on the fatty acid composition\cite{17}. These results suggest that the difference in the interfacial properties of the various MEL highly depends on the structure of both the acetyl and fatty acid moieties.

The above MEL producers showed high MEL production yields when vegetable oil was supplied as the sole carbon source. Fatty acid metabolism in P. antarctica has been previously investigated\cite{17, 18}, and the evidence suggested that MEL biosynthesis occurs through the newly recognized “chain-shortening pathway”, such as the partial $\beta$-oxidation system known in mammalian peroxisomes\cite{19}. Studies on Ustilago maydis shed light on the involvement of four genes (emt1, erythritol/mannose transferase; mac1 and mac2, acyl-transferases; mat1, acyl-transferase) in MEL biosynthesis\cite{20, 21}. The intermediates in the “chain-shortening pathway” would transfer to the mannose moiety by the reaction of acyl-transferases, mac1 and mac2. Therefore, the composition of the oil supplied as well as the substrate specificity of the acyl-transferases probably decides the fatty acid composition of MELs.

Recently, we found that the presence of unsaturated fatty acids in MEL could potentially enhance their anti-oxidative activity\cite{22}. Thus, the development of MEL possessing large amounts of unsaturated fatty acids became attractive for their use as new anti-aging skin care ingredients.

Cuttlefish is one of the most popular seafood in Japan, but the oil extracted from its viscera is usually discarded. On the other hand, cuttlefish oil is very rich in polyunsaturated fatty acids, as for instance docosahexaenoic acid (DHA, C22:6n3) and eicosapentaenoic acid (EPA, C20:5n3)\cite{23}, and thus, it seems to be a potential bio-resource for the fermentation industry. Although cuttlefish oil would enable the production of MEL enriched in unsaturated fatty acids, studies regarding their synthesis production from this oil have not been published yet.

Here, we attempted to obtain MELs containing polyunsaturated fatty acids from P. churashimaensis OK96 by using cuttlefish oil as the sole carbon source. Then, we investigated the interfacial and self-assembling properties of the obtained MEL-A containing hexadecatetraenoic acid ($C_{16:4}$). We also evaluated the anti-oxidative activity of the newly identified MEL.

2 EXPERIMENTAL

2.1 Yeast strains and growth medium

P. rugulosa JCM 10323 and P. parantarctica JCM 11752 were obtained from RIKEN BioResource Center (Saitama, Japan). P. tsukubaensis NBRC 1940 and U. scitaminea NBRC 32730 were obtained from the National Institute of Technology and Evaluation of Japan (Chiba, Japan). P. graminicola CBS 10092, P. shanxiensis CBS 10075, and P. siamensis CBS 9960 were obtained from Centraal bureau voor Schimmel cultures (Utrecht, The Netherlands). P. antarctica T-34, P. hubeiensis KM59, and P. churashimaensis OK96 were from our laboratory stock\cite{10, 14, 24}. Stock cultures were cultivated for 3 d at 25°C on YM agar plate containing 1% glucose, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract, and 3.0% agar. They were then stored at 4°C and renewed every 14 d. Cuttlefish (Sepiella maindroni de Rochebruns) oil was a gift from Nippon Chemical Feed Co., Ltd. (presently known as Hokkaido Fine Chemicals Co., Ltd.), Hakodate, Japan and contained 15.8% palmitic acid ($C_{16:0}$), 14.9% oleic acid ($C_{18:1}$), 13.2% eicosapentaenoic acid (EPA, C20:5n3), and 15.4% docosahexaenoic acid (DHA, C22:6n3).
2.2 Medium preparation and culture conditions

Seed cultures were prepared by inoculating the cells that were previously grown on slants into test tubes containing the YM medium (1% glucose, 0.3% peptone, 0.3% yeast extract, and 0.3% malt extract) at 25°C on a reciprocal shaker at 200 strokes/min for 2 d. Seed cultures were then transferred to 300-mL Erlenmeyer flasks containing 30 mL of medium (50 g/L of cuttlefish oil, 0.3% NaNO₃, 0.03% MgSO₄, 0.03% KH₂PO₄, 0.1% yeast extract, pH 6.0), and then incubated at 25°C on a rotary shaker (200 rpm) for 7 d. The culture broth (35 mL) were inoculated in 700 mL of the same medium in a 1.5-L jar fermenter (TBR-2-3; Sakura, Tokyo, Japan) and shaken at 600 rpm at 25°C, 1.5 L/min.

2.3 Isolation of MEL

The produced MEL was extracted from the cultures with equal volumes of ethyl acetate. The extracts were analyzed by thin-layer chromatography (TLC) on silica plates (Silica gel 60F; Wako, Osaka, Japan) with a solvent system consisting of chloroform/methanol/7N ammonia solution (65:15:2, by volume). The compounds on the plates were localized by charring at 110°C for 5 min after spraying the anthrone/sulfuric reagent as previously described (25). Purified MEL fractions containing MEL-A, MEL-B, and MEL-C, prepared as previously reported (25), were used as standards.

2.4 Purification of MEL

The ethyl acetate fractions were separated and evaporated in order to concentrate the glycolipids that were later re-dissolved in chloroform. Solubilized MEL were then purified by reverse-phase chromatography using a silica gel column (Wako-gel C-200; Wako, Osaka, Japan) and a gradient elution protocol consisting of chloroform/acetone (10:0 to 3:0, v/v) mixtures (26). The purified MEL were used for the following experiments.

2.5 High-performance liquid chromatography (HPLC)

The quantification of MEL and cuttlefish oil was carried out by HPLC on a reverse-phase silica gel column (Inertsil SIL 100A 5 μm, 4.6 × 250 mm; GL Science, Tokyo, Japan) equipped with a low-temperature evaporative light scattering detector (ELSD-LT; Shimadzu, Kyoto, Japan), by using a gradient solvent program consisting of various proportions of chloroform and methanol (from 100:0 to 0:100, v/v) at a flow rate of 1 mL/min (26). The HPLC analysis was based on the standard curve obtained by using the purified MEL fraction prepared as previously reported (25).

2.6 Structural analysis

The structure of the purified MEL dissolved in CDCl₃ was confirmed by ¹H and ¹³C nuclear magnetic resonance (NMR) with a Varian INOVA 400 (400 MHz) at 30°C. The molecular weight of the purified MEL was measured by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) (Voyager-DE PRO; Applied Biosystems, IL, USA) on a α-cyano-4-hydroxyxynamic acid matrix.

The fatty acid profile of the purified MEL was examined as previously described (25). Methyl ester derivatives of the fatty acids were prepared by mixing the purified MEL-A (10 mg) with 5% HCl-methanol (1 mL). After quenching the reaction with water (1 mL), the methyl ester derivatives were extracted with n-hexane, and then analyzed by gas chromatography-mass spectrometry (GC-MS) (Hewlett Packard 6890 and 5973N; Agilent Technologies, DE, USA) using a TC-WAX column (GL Science, Tokyo, Japan) with a temperature gradient programmed from 90°C (held for 3 min) to 240°C at a speed of 5°C/min.

2.7 Determination of surface tension

The surface tension of the purified MEL was determined at 25°C by the Wilhelmy method (27), which was performed using a Wilhelmy-type tension meter (CBVP-A3; Kyowa Interface Science Co., Ltd., Saitama, Japan).

2.8 Water-penetration scanning technique

To examine the lyotropic liquid crystalline phase behavior of the purified MEL, the water-penetration scanning technique was used as previously reported (25). Briefly, the scans were carried out by using a polarized optical microscope (ECLIPSE E-600; Nikon, Tokyo, Japan) carrying crossed-polarizing filters and equipped with a charge-coupled device camera (DS-SM; Nikon, Tokyo, Japan). The birefringent textures observed with the optical microscope allowed for the assignment of the particular lyotropic phase types to the different samples.

2.9 Confocal laser scanning microscopy (CLSM)

Confocal micrographs were recorded with a LSM 5 PASCAL (Zeiss, Jena, Germany), equipped with a 200 × objective lens with a numerical aperture of 0.5. Helium-neon laser excitation at 543 nm was used in combination with a 560 long-pass filter and a HFT 543 dichroic mirror. The assemblies were made visible by solubilizing hydrophobic Nile red into the colloidal structures as a fluorescence probe (MEL: Nile red = 700: 1).

2.10 DPPH free radical-scavenging assay

Purified MEL were dissolved in 50 μL of ethanol (1.3, 2.5, 5, and 10 mg/mL final concentrations), and mixed with 50 μL of Tris-HCl buffer (pH 7.4) on a 96-well microplate. After the mixture has been pre-incubated for 10 min at 25°C, 0.3 mM DPPH ethanol solution (50 μL) was added, and the mixture was incubated for 40 min at 25°C. Arbutin (final concentrations, 0.13, 0.25, 0.5, and 1 mg/mL) and pure ethanol were used as positive and negative controls, respectively. The absorbance of the mixtures was measured using a microplate reader (J. Oleo Sci. 62, (2013) 319-327).
of 50 g/L of cuttlefish oil at 25°C. Interestingly, in the case of *P. churashimaensis* OK96, the fatty acid fraction of MEL-A from cuttlefish oil was characterized by many unknown GC peaks, compared to the case of soybean oil (Fig. 3).

Of these unknown peaks, the largest peak (peak A; approximately 23.6% of the total unsaturated fatty acids, 7.7% of the total fatty acids) at 12.11 min was identical with hexadecatetraenoic acid on the basis of the mass spectrum pattern and the molecular ion peak at 262 $^{19}$O. Furthermore, peak A disappeared after hydrogenation (data not shown). These results strongly indicate that *P. churashimaensis* OK96 is able to produce MEL-A incorporating the hexadecatetraenoic acid when cuttlefish oil is provided as a substrate.

The chemical shifts of the hydrophilic moiety of the MEL-A produced from cuttlefish oil were as follows: mannose H-1' at 4.74 ppm (s), H-2' at 5.52 ppm (d), H-3' at 5.08 ppm (dd), H-4' at 5.26 ppm (t), H-5' at 3.72 ppm (m), H-6' at 3.83 ppm (dd), erythritol H-1 at 3.56-3.86 ppm (m), H-2 at 3.56-3.86 ppm (m), H-3 at 3.60-3.85 ppm (m), H-4a at 3.83 ppm (dd), and H-4b at 4.00 ppm (dd). The acetyl group (–OCH3, at the C6' position of mannose) peak at 2.13 ppm (s). According

3 RESULTS

3.1 Production of MEL from cuttlefish oil by different ustilaginomycetous strains

To investigate the production of MEL using cuttlefish oil as the sole carbon source, ten ustilaginomycetous strains that are known to produce MEL (*P. antarctica* T-34, *P. rugulosa* JCM 10323, *P. parantarctica* JCM 11752, *P. churashimaensis* OK96, *P. tsukubaensis* NBRC 1940, *U. scitaminea* NBRC 32730, *P. hubetensis* KM-59, *P. graminicola* CBS 10092, *P. siamensis* CBS 9960, and *P. shanxiensis* CBS 10075) were cultured in a medium containing 50 g/L of cuttlefish oil at 25°C for 7 d.

After the cultivation, an equal volume of ethyl acetate was added to the culture medium and the obtained ethyl acetate fractions were spotted on a TLC plate. Nine of the ten strains provided the blue spots corresponding to MEL-A, MEL-B, and/or MEL-C, with the exception of *P. tsukubaensis* NBRC 1940.

To quantify the amounts of produced MEL, the ethyl acetate extracts were analyzed by HPLC using a reverse-phase column (Fig. 2). Among the nine strains, *P. parantarctica* JCM 11752 showed the highest production yield of MEL from cuttlefish oil with a total of 38.8 g/L of MEL after the cultivation for 7 d.

3.2 Fatty acid composition of MEL produced from cuttlefish oil

To determine their fatty acid composition, purified MEL were degraded under acidic conditions, and the fatty acid fraction was analyzed by GC/MS (Table 1). As a comparison, MEL produced from vegetable oils such as soybean, olive, and safflower oil, which are mainly composed of C18 fatty acids, were also analyzed.

Scavenging activity (%) = $\frac{(A_{tb517} - A_{cb517})}{(A_{tb517} - A_{cb517})} \times 100$ (1)
Characterization of Mannosylerythritol Lipids Containing Hexadecatetraenoic Acid Produced from Cuttlefish Oil by Pseudozyma churashimaensis OK96

Table 1 Fatty acid profiles of MELs produced from cuttlefish oil and vegetable oils.

<table>
<thead>
<tr>
<th>Fatty acid type</th>
<th>Composition (%)</th>
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<tr>
<td></td>
<td>MEL-A</td>
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<tr>
<td>P. antarctica</td>
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<tr>
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<td>JCM1752</td>
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<td>OK96</td>
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<td>P. shansiensis CBS10075</td>
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<td>Cutt. Soy.</td>
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<td>Cutt. Soy.</td>
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<td>Oliva</td>
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<td>18.9</td>
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<td>29.4</td>
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<td>44.6</td>
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<td>29.5</td>
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<td>25.7</td>
<td>44.8</td>
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<td>27.1</td>
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Cutt., cuttlefish oil; Soy., soybean oil; Olive, olive oil; Saff., safflower oil
* Ref. 25; † Ref. 7; ‡ Ref. 12; § Ref. 13; ‖ Ref. 11.

3.3 Production of MEL from cuttlefish oil by P. churashimaensis OK96

We also investigated MEL production from cuttlefish oil by P. churashimaensis OK96 by using a 1.5-L jar fermenter. Figure 4 shows a typical time course of MEL production by the yeast using 750 mL of medium containing 50 g/L of cuttlefish oil. After cultivation at 25°C for 7 d, the total amount of MEL reached 9.6 g/L, corresponding to 0.19 g per gram of supplied cuttlefish oil. Consequently, in the jar fermenter, the production yield of MEL by P. churashimaensis OK96 increased more than 1.7-fold compared to the conventional shake-flask culture.

3.4 Surface-active properties of MEL-A produced from cuttlefish oil by P. churashimaensis OK96

Due to the presence of hexadecatetraenoic acid in the MEL-A from cuttlefish oil, its hydrophobic structure is considerably different from that of conventional MEL that instead mainly contain two medium-length fatty acids (C14 to C16) (Table 1). We thus determined surface tension of the MEL-A by exploiting the Wilhelmy method. The estimated CMC and surface tension at CMC (γCMC) of the MEL-A from cuttlefish oil were 5.7 × 10⁻⁶ M and 29.5 mN/m, while those of MEL-A from soybean oil were 2.7 × 10⁻⁶ M and 27.7 mN/m, respectively. When cuttlefish oil was provided as substrate, MEL-A retained an excellent surface tension-lowering activity, although the fatty acid composition was quite different from that of conventional MEL previously described.

3.5 Self-assembling properties of MEL-A produced by P. churashimaensis OK96 from cuttlefish oil

We further investigated the self-assembling properties of MEL-A in aqueous solution by the water penetration scanning method. Figure 5 displays MEL-A water penetration scans obtained with (bottom, POL) and without (upper,
DIC crossed-polarizing filters. In the case of MEL-A produced from soybean oil, the photographs clearly indicated four different regions representing water (W), myelines, lamellar phase (Lα) and nest surfactant phase (S), and showed an immediate formation of lamellar phase and myelines over a broad range of concentrations. In contrast, no anisotropic regions were observed in MEL-A produced from cuttlefish oil.

The self-assembled structures of MEL-A (6.8 wt%) in aqueous solution were then investigated by CLSM using Nile Red as a fluorescence probe. Red globular particles with a diameter of approximately 20 μm were observed (Fig. 6). This structure seemed to be identical to the isotropic L₃ phase observed in the case of MEL-A produced by *P. antarctica* from soybean oil. Thus, the increased amount of unsaturated fatty acids in MEL-A seems to considerably affect its self-assembling property.

### 3.6 Anti-oxidative activity of MEL-A produced by *P. churashimaensis* OK96 from cuttlefish oil

To estimate the antioxidant properties of MEL-A incorporating the hexadecatetraenoic acid, the DPPH radical-scavenging activity of the glycolipid was evaluated and compared with that of MEL-A produced from soybean oil. In this study, arbutin, which is well known to act as a strong scavenger, was used as the positive control.

Both MEL-A showed a concentration-dependent scavenging activity (Fig. 7). However, the scavenging activity of the MEL-A from cuttlefish oil (IC₅₀ = 14.0 mg/mL) was 2.1-fold higher than that of MEL-A from soybean oil (IC₅₀ = 29.4 mg/mL). Accordingly, we concluded that the polyunsaturated hexadecatetraenoic acid is likely to improve MEL-A anti-oxidative properties.
In this study, we achieved the production of MEL-A, a new type of MEL possessing hexadecatetraenoic acid ($C_{16:4}$, by feeding *P. churashimaensis* OK96 with cuttlefish oil as the sole carbon source, and demonstrated that such MEL-A possesses different self-assembling and antioxidative properties compared to the conventional MEL. To our best knowledge, this is the first report on MEL bearing polyunsaturated fatty acids.

The conventional representative MEL producers such as *P. antarctica*, *P. rugulosa*, and *P. parantarctica*, were able to produce large amounts of MEL from cuttlefish oil with yields over 30 g/L. However, the amount of unsaturated fatty acids in MEL from cuttlefish oil was significantly lower than that of MEL produced from soybean oil. In contrast, *P. churashimaensis* OK96, which was recently isolated from sugarcane leaf as a novel MEL producer[^4^], produced MEL consisting of large amounts of unsaturated fatty acids (40% of the total fatty acids content). In addition, the presence of unsaturated fatty acids in MEL significantly increased when cuttlefish oil was used instead of soybean oil.

Based on our previous studies[^1^], since most of the fatty acids in MEL are β-oxidation intermediates of the fatty acids supplied as carbon source, their chain length is shorter than that of the supplied fatty acids. Indeed, in the case of the strains used in this work, the fatty acids incorporated in MEL were constituted by $C_{10}$ chains, which are much shorter than those of their precursors (DHA and EPA). On the other hand, the fatty acids contained in MEL from *P. churashimaensis* OK96 mainly carried $C_{16}$ chains. In the case of $C_{16}$ acids, it is likely that the double bonds in DHA and EPA were preserved after β-oxidation and were later incorporated into MEL by the acyl-transferase. Considering the structures of DHA ($C_{22:6}$) and EPA ($C_{20:5}$), it seems reasonable that they would provide hexadecatetraenoic acid ($C_{16:4}$) after being processed by two or three cycles of β-oxidation. Nevertheless, detailed biochemical studies should be conducted on *P. churashimaensis* OK96 to shed light on the biosynthesis mechanism of MEL-A.

In the present study, we also tried to improve the production efficiency of MEL from cuttlefish oil by using *P. churashimaensis* OK96. With a jar fermenter, the yield became 1.7-fold higher compared to the shake-flask culture; the yield, productivity, and yield coefficient were 9.6 g/L, 1.4 g/L/d, and 0.19 g per gram of substrate, respectively. We previously reported that MEL production by *P. aphidis* is highly enhanced over 165 g/L on a jar fermenter by feeding of soybean oil and additional supplements (glucose, sodium nitrate, and yeast extract)[^2^]. Thus,
further optimization of the culture medium might increase the MEL production yield from a particular oil, and also facilitate the oil utilization during the microbial processes.

In addition to the excellent surface activity, another interesting feature of MEL-A is the formation of different lyotropic liquid crystalline phases in aqueous solutions. The conventional MEL-A produced from soybean oil by *P. antarctica* was reported to self-assemble into a variety of liquid crystalline phases, including sponge (L₃), bi-continuous cubic (V₂), and lamella (L₆). The MEL-A produced from soybean oil by *P. churashimaensis* OK96 also formed at least two different phases such as myeline and lamella (L₆). In contrast, based on CLSM measurements, MEL-A from cuttlefish oil did not generate anisotropic phases, but formed spherical droplets similar to an isotropic L₃ phase. This result is probably linked to the difference in the amounts of polyunsaturated fatty acids between the two MEL-A.

We also recently found that different types of MEL exhibit anti-oxidative properties leading to anti-aging. In particular, MEL-C including diene unsaturated fatty acids shows higher protective activity against oxidative stress than arbutin in fibroblast cells. Here, we also unveiled that the radical-scavenging activity of MEL-A from cuttlefish oil was 2.1-fold higher than that of MEL-A from soybean oil. Considering these results, it is reasonable to conclude that a higher amount of polyunsaturated fatty acids is likely to enhance MEL anti-oxidative activity.

Previously, we reported that MEL show ceramide-like skin care and hair care properties due to their moisturizing action, and induce proliferation in cultured human skin fibroblast cells leading to regeneration of the skin itself. These results strongly suggest that MEL incorporating unsaturated fatty acids could potentially become new skin care ingredients bearing moisturizing as well as anti-oxidative properties.

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