Mannosylerythritol Lipids: Production and Applications

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Abstract: Mannosylerythritol lipids (MELs) are a glycolipid class of biosurfactants produced by a variety of yeast and fungal strains that exhibit excellent interfacial and biochemical properties. MEL-producing fungi were identified using an efficient screening method for the glycolipid production and taxonomical classification on the basis of ribosomal RNA sequences. MEL production is limited primarily to the genus Pseudozyma, with significant variability among the MEL structures produced by each species. Outside of Pseudozyma, one recently isolated strain, Ustilago scitaminea, has been shown to exhibit abundant MEL-B production from sugarcane juice. Structural analyses of these compounds suggest a role for MELs in numerous cosmetic applications. MELs act as effective topical moisturizers and can repair damaged hair. Furthermore, these compounds have been shown to exhibit both protective and healing activities, to activate fibroblasts and papilla cells, and to act as natural antioxidants. In this review, we provide a brief summary of MEL research over the past few decades, focusing on the identification of MEL-producing fungi, the structural characterization of MELs, the use of alternative compounds as a primary carbon source, and the use of these compounds in cosmetic applications.

Key words: glycolipid, biosurfactant, mannosylerythritol lipid, yeast, sugarcane, cosmetics

1 MANNOSYLERITHITOL LIPIDS (MELs)

MELs are long chain fatty acids that contain either 4-O-β-D-mannopyranosyl-erythritol or 1-O-β-D-mannopyranosylerythritol as the hydrophilic head group, which is attached to a variety of fatty acids as the hydrophobic chain (Fig. 1). These compounds are known to act as functional glycolipids, and are produced almost exclusively by yeast strains of the genus Pseudozyma1-3. MELs exhibit excellent interfacial properties4, as well as a variety of other biochemical functions, including the induction of differentiation in mammalian cells and the binding of various classes of immunoglobulins5. As with many lipids, differences in chemical structure directly affect their biological activity; for example, MEL-A, a diacetylated MEL, has been shown to strongly increase the efficiency of gene transfection using cationic liposomes6,7. Furthermore, certain MELs have been shown to possess anti-inflammatory properties, such as inhibiting the secretion of inflammatory mediators from mast cells8-12. When combined with their high biodegradability and relative ease of production, these compounds hold tremendous promise for use in a wide range of applications, including food production, cosmetics, and pharmaceuticals5,13.

Here, we provide a brief summary highlighting recent advances in MEL research, including the identification of MEL-producing fungi, structural characterizations of MELs, the use of alternative compounds as a primary carbon source, and the use of MELs in cosmetic applications.

2 IDENTIFICATION OF MEL-PRODUCING FUNGI

To identify a wide variety of MEL structures, we first sought to develop a rapid and efficient method for detecting MEL production on the basis of decreasing surface tension in cultures containing biosurfactants. The surface tension of each culture was assessed by evaluating the diameter of droplets on the surface of a hydrophobic film (Fig. 2)14. MELs were then detected by thin-layer chroma-
Fig. 1 Chemical structure of glycolipids produced by yeast strains of the genus *Pseudozyma*. (a) Tri-acylated MEL. (b) di-acylated MEL. (c) mono-acylated MEL. (d) diastereomer type of MEL. (e) mono-acylated and tri-acylated MEL. (f) mannosyl-mannitol lipid. (g) mannosyl-arabitol lipid. (h) mannosyl-ribitol lipid. MEL-A: R₁=Ac, R₂=Ac; MEL-B: R₁=Ac, R₂=H; MEL-C: R₁=H, R₂=Ac; MEL-D: R₁=H, R₂=H; n = 4-16; m = 6-16.

Medium

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Glycerol</th>
<th>Soybean oil</th>
<th>Carbon source</th>
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Fig. 2 The shape of the culture supernatant containing MELs (modified from Morita *et al.*, 2006)⁹. The surface tension of the culture medium of *P. antarctica* grown with glucose, glycerol, and soybean oil were visualized on the hydrophobic surface of Parafilm M, reported previously.
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tography (TLC). This novel screening method resulted in the identification of numerous MEL-producing fungi, as well as many previously undescribed MEL structures. For example, an MEL-producing species of the genus Pseudozyma, Pseudozyma churashimaensis, isolated from sugarcane, was found to produce not only MEL-A as the major product, but also two novel compounds, monoacylated and triacetylated MELs, as the minor fraction (Fig. 1e)\(^{\text{15}}\). Alternatively, the smut fungus Ustilago scitaminea, also isolated from sugarcane, was found to produce MEL-B\(^{\text{16}}\). Other fungi shown to produce diastereomers of MEL-B include Pseudozyma tsukubaensis strains 1D9, 1D10, 1D11, and 1E5, all of which were isolated from Perilla frutescens leaves\(^{\text{17}}\). Fifteen additional strains of MEL-producing fungi, representing a variety of Pseudozyma yeasts, were isolated from various vegetables and fruits using this new screening method\(^{\text{18}}\).

### 3 TAXONOMY OF MEL-PRODUCING FUNGI

The vast majority of MEL-producing fungi belong to the genus Pseudozyma. The first-known MEL producer, *Pseudozyma antarctica* T-34, was first identified over two decades ago; it produces mainly MEL-A, along with smaller amounts of MEL-B and MEL-C\(^{\text{19}}\). More recently identified MEL-producing fungi include *Pseudozyma rugulosa*, *Pseudozyma aphidis*, *Pseudozyma parantarctica*, *P. tsukubaensis*, *Pseudozyma fusiformata*, *Pseudozyma graminicola*, *Pseudozyma shanxiensis*, *Pseudozyma siamensis*, and *Pseudozyma crassa*\(^{\text{20–24}}\), in addition to the smut fungus *Ustilago cynodontis*, which produces primarily MEL-C when grown in the presence of vegetable oil\(^{\text{25}}\). Not surprisingly, the taxonomic distribution of these fungi is strongly associated with MEL production (Fig. 3). Fungi that produce mainly MEL-A, including *P. antarctica*, *P. aphidis*, *P. rugulosa*, and *P. parantarctica*, are closely linked within a discrete branch of the phylogenetic tree. A producer of MEL-A diastereomers, *P. crassa*, also clustered closely with the MEL-A producers, albeit in a separate taxonomic branch. The producer of a diastereomeric MEL-B,

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**Fig. 3** Molecular phylogenetic tree constructed using ITS1, 5.8S rRNA gene, and ITS2 sequences of the genus *Pseudozyma* and *Ustilago* (from Morita et al., 2009)\(^{\text{11}}\). The DDBJ/GenBank/EMBL accession numbers are indicated in parentheses.

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P. tsukubaensis, was independently positioned in the tree, while MEL-C-producing fungi were scattered throughout the tree, indicative of less conservation between these species. Additional genetic studies will be necessary to better characterize the structures of less common MEL variants, and to determine the relationship between the fungi that produce them.

4 STRUCTURAL DIVERSITY OF MELs

As described previously, P. antarctica, P. aphidis, P. rugulosa, and P. parantarctica are the largest producers of MELs, mainly MEL-A (a diacetylated MEL) along with smaller percentages of MEL-B and MEL-C (Fig. 1b). These fungi can produce >100 g/L of MELs using vegetable oil as a substrate. P. tsukubaensis selectively produces a diastereomer of MEL-B. The sugar moiety found in this alternative MEL-B consists of 1-O-β-D-mannopyranosyl-erythritol (Fig. 1d), an isomer of the traditional 4-O-β-D-mannopyranosyl-erythritol found in other MELs. Pseudozyma tsukubaensis produces diastereomers of MEL-A, MEL-B, and MEL-C, all of which are stereochemically distinct from conventional MELs. Pseudozyma hubeiensis, P. graminicola, and P. shanxiensis produce MEL-C exclusively.

5 PRODUCTION OF MEL-B USING SUGARCANE JUICE AS THE PRIMARY CARBON SOURCE

Vegetable oils are often the best carbon source for MEL production; however, the use of these oils in place of more hydrophilic compounds presents significant challenges for many downstream applications due to the presence of chemical by-products such as free fatty acids, monoglycerides, and diacylglycerols. Significant attention has therefore been placed on finding a suitable water-soluble carbon source (e.g., glucose or glycerol) for use in downstream applications. Among the MEL-producing fungi, we identified a smut fungus, U. scitaminea NBRC32730, which was able to produce large amounts of MEL-B using only sucrose, glucose, fructose, or mannose as the sole carbon source. This strain was also able to produce MEL-B using sugarcane juice supplemented with urea as the main nutrient source, reaching a maximum yield of 25 g/L (Fig. 4). Furthermore, TLC revealed a single spot corresponding to MEL-B, indicative of high baseline purity without the need for additional purification steps.

6 COSMETIC APPLICATIONS

6.1 Moisturizing activity

In the course of our research, we assumed that MELs are able to express the moisturizing activity similar to ceramide-3 (Fig. 5a), an essential component of the stratum corneum, because both of the lipids consist of two fatty acids and sugar moieties, and are capable of forming various lyotropic liquid crystals, including the lamellar phase. As previously reported, the moisturizing activity was evaluated using a three-dimensional cultured human skin model, TESTSKIN (Toyobo, Japan) based on the cell viability and the sodium dodecyl sulfate (SDS)-damaged human skin cells were prepared, and then the effects of different lipids on viability of the SDS-damaged cells. Consequently, MELs were found to exhibit moisturizing properties similar to those of ceramide-3 (Fig. 5b). Furthermore, the diastereomeric form of MEL-B exhibited excellent water retention properties, increasing the water content within the stratum corneum, while visibly suppressing perspiration on the skin surface. These results indicate that MELs may hold promise as an alternative, cost-effective skincare ingredient, capable of replacing higher priced ingredients such as natural ceramides.

6.2 Repair of damaged hair

The ceramide-like properties of MELs were also found to extend into the realm of hair care. Ceramides are present not only in the stratum corneum, but also the cuticle of hair, protecting and repairing hair fibers in the face of a variety of environmental stresses. Electron microscopy showed that both MEL-A and MEL-B were able to repair fine cracks on the surface of artificially damaged hair in a manner equivalent to that of ceramides. Furthermore, MEL treatment increased the overall strength and sustained the average friction coefficient of damaged hair. These results suggest that MELs are a useful ingredient in many downstream applications due to the presence of chemical by-products such as free fatty acids, monoglycerides, and diacylglycerols. Significant attention has therefore been placed on finding a suitable water-soluble carbon source (e.g., glucose or glycerol) for use in downstream applications. Among the MEL-producing fungi, we identified a smut fungus, U. scitaminea NBRC32730, which was able to produce large amounts of MEL-B using only sucrose, glucose, fructose, or mannose as the sole carbon source. This strain was also able to produce MEL-B using sugarcane juice supplemented with urea as the main nutrient source, reaching a maximum yield of 25 g/L (Fig. 4). Furthermore, TLC revealed a single spot corresponding to MEL-B, indicative of high baseline purity without the need for additional purification steps.

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6.3 Activation of fibroblasts and papilla cells

In addition to their reparative functions, MELs have been shown to activate fibroblasts and papilla cells, leading to follicle formation and hair growth via transdifferentiation of the adult epidermis. Treatment with MEL-A markedly increased the viability of both fibroblasts and papilla cells by more than 150% relative to controls. Consequently, MEL-A may hold promise as a hair growth treatment on the basis of its cell activation properties.

6.4 Antioxidative effects

Protection against oxidative stress is essential for long-term skin health, and is therefore an important topic in the cosmetics industry. MELs harboring unsaturated fatty acids in the hydrophobic portion of the molecule exhibit both 1,1-diphenyl-2-picryl hydrazine radical- and superoxide anion-scavenging activities, albeit at levels below that of arbutin, a strong scavenger of reactive oxygen species. Further investigation into the antioxidant properties of MELs using cultured human skin fibroblasts under H₂O₂-induced oxidative stress revealed significant cytoprotective activity similar to that of arbutin. MELs may therefore be useful as an anti-aging ingredient in skin care products due to their combination of both antioxidant and cytoprotective effects.

7 FURTHER PERSPECTIVES

Naturally occurring extracellular glycolipids, including MELs, have been investigated for their use as environmentally friendly biosurfactants with excellent interfacial and biochemical properties. To facilitate their use in a wide

| Table 1  MEL producers and their products (modified from Morita et al., 2013) |
|----------------------------------|------------------|------------------|------------------|------------------|
| Microbial producers             | Carbon sources   | Glycolipids       | Yield (g/L)      | cmc (M)          | gcme (mN/m)      | References   |
| Pseudozyma aphidis              | Soybean oil and glucose | MEL-A (main), MEL-B, MEL-C | 165^a,b         | ND              | 26.2             | 2, 28, 29 |
| Pseudozyma atarctica           | Soybean oil      | MEL-A (main), MEL-B, MEL-C | 40^c           | 2.7 × 10⁻⁸      | 28.4             | 2, 4, 19   |
|                                | Soybean oil      | MEL-B (purified)  | 10             | 4.5 × 10⁻⁸      | 28.2             | 4, 19      |
|                                | n-Alkane         | MEL-A (main), MEL-B, MEL-C | 140^d         | ND              | ND              | 27         |
|                                | Glucose          | Mono-acylated MEL (purified) | 1.3            | 3.6 × 10⁻⁸      | 33.8             | 36         |
|                                | Soybean oil      | Try-acylated MEL (purified) | ND            | ND              | ND              | 34, 35     |
| Pseudozyma charashimaensis     | Soybean oil      | Mono-acylated/tri-acetylated MEL (purified) | 3.8^e          | 1.7 × 10⁻⁸      | 29.2             | 15         |
| Pseudozyma crassa              | Oleic acid and glucose | Diastereomer MEL-A (main), MEL-B, MEL-C | 4.6^b          | 5.2 × 10⁻⁸      | 26.5             | 20         |
| Pseudozyma fujiformata         | Soybean oil      | MEL-A (main), MEL-B, MEL-C | 4^f           | ND              | ND              | 2         |
| Pseudozyma graminicola         | Soybean oil      | MEL-A, MEL-B, MEL-C (main) | 9.6^g          | 4.0 × 10⁻⁸      | 24.2             | 22         |
| Pseudozyma huebeensis          | Soybean oil      | MEL-A, MEL-B, MEL-C (main) | 76.3^h,i       | 6.0 × 10⁻⁸      | 25.1             | 32         |
| Pseudozyma parantarctica       | Soybean oil      | MEL-A (main), MEL-B, MEL-C | 106.7^j, k     | ND              | ND              | 2, 34      |
|                                | Glucose          | Mono-acylated MEL (purified) | 1.2            | ND              | ND              | 36         |
|                                | Soybean oil      | Try-acylated MEL (purified) | 22.7           | ND              | ND              | 34         |
|                                | Olive oil and mannitol | Mannosyl-mannitol lipids (purified) | 18.2          | 2.6 × 10⁻⁸      | 24.2             | 35         |
|                                | Olive oil and arabitol | Mannosyl-arabitol lipids (purified) | ND            | 1.5 × 10⁻⁸      | 24.2             | 39         |
|                                | Olive oil and ribitol | Mannosyl-ribitol lipids (purified) | ND            | 1.2 × 10⁻⁸      | 23.7             | 39         |
| Pseudozyma rugulosa            | Soybean oil and erythritol | MEL-A (main), MEL-B, MEL-C | 142^l,m           | ND              | ND              | 2, 21      |
| Pseudozyma shanxiensis         | Soybean oil      | Try-acylated MEL (purified) | ND            | ND              | ND              | 35         |
| Pseudozyma siamensis           | Safflower oil    | MEL-B, MEL-C (main) | 18.5^n         | 4.5 × 10⁻⁸      | 30.7             | 24         |
| Pseudozyma tsukubaeensis       | Soybean oil      | Diastereomer MEL-B | 73.1^o,p       | 3.1 × 10⁻⁸      | 26.1             | 2, 17, 30 |
|                                | Castor oil       | Diastereomer MEL-B containing a hydroxy fatty acid | 22           | 2.2 × 10⁻⁸      | 28.5             | 37         |
| Ustilago cynodontis             | Soybean oil      | MEL-C              | 1.4            | ND              | ND              | 25         |
| Ustilago maydis                | Sunflower oil    | MELs and cellobiose lipids | 30^q          | ND              | ND              | 50         |
| Ustilago scitaminea            | Sugarcane juice  | MEL-B              | 25.1           | 3.7 × 10⁻⁸      | 25.2             | 16         |

a As a mixture of MELs
b Feeding using resting cells
c Large scale production with jet-fermenter
d As a mixture of MELs and cellobiose lipids
ND: no data

1.1-diphenyl-2-picrylhydrazine radical- and superoxide anion-scavenging activities, albeit at levels below that of arbutin, a strong scavenger of reactive oxygen species. Further investigation into the antioxidant properties of MELs using cultured human skin fibroblasts under H₂O₂-induced oxidative stress revealed significant cytoprotective activity similar to that of arbutin. MELs may therefore be useful as an anti-aging ingredient in skin care products due to their combination of both antioxidant and cytoprotective effects.

7 FURTHER PERSPECTIVES

Naturally occurring extracellular glycolipids, including MELs, have been investigated for their use as environmentally friendly biosurfactants with excellent interfacial and biochemical properties. To facilitate their use in a wide
Fig. 4  Production of MEL-B from sugarcane juice supplemented with urea by *Ustilago scitaminea* NBRC 32730 (modified from Morita *et al*., 2011). (a) The cells of strain NBRC 32730 were cultivated in 700 mL of the sugarcane juice supplemented with urea (1 g/L) in a jar-fermenter (1.5 L) at 25°C for 7 d. MEL-B was extracted from the cultured medium using an equal amount of ethyl acetate, and quantified by HPLC (closed circle). The amounts of sugars were quantified by HPLC after filtration: sucrose (closed square), glucose (closed diamond), fructose (open square). (b) After 7 d cultivation, the ethyl acetate extract from the culture medium showed a single peak corresponding to MEL-B on HPLC analysis. The purified MELs (MEL-A, MEL-B, and MEL-C) were used as the standard. The retention time (min) of each peak is given in parentheses. (c) The extract also showed a single spot corresponding to MEL-B on TLC. The purified MELs (MEL-A, MEL-B, and MEL-C) were used as the standard.

Fig. 5  Moisturizing property of MELs (modified from Morita *et al*., 2009). (a) Chemical structure of ceramide 3. (b) Relative viability of the cultured skin cells treated with SDS. The cultured skin cells were treated with 1% SDS, washed out the SDS solution, and then re-treated with MELs-A dissolved in olive oil. The viability of cells was determined with a colorimetric method (MTT assay) at 570 nm. Ceramide was used as the positive control. -SDS: non-treated with SDS, +SDS: treated with SDS.
range of industrial applications, we developed an efficient method for the detection and isolation of MEL-producing fungi, from which we were able to expanded both the structural and functional varieties of MELs. Since then, our investigations have focused primarily on the interfacial properties and practical characteristics of MELs, with the goal of using MELs in cosmetic applications. From this work, we have been able to dramatically reduce the production cost of MELs through the use of an improved fermentation process, and the identification of fungal strains capable of using raw sugarcane juice as the main carbon source. Further decreases in the overall production cost will be necessary for the expanded use of MELs outside of the cosmetics industry, as described previously. Additional investigations regarding the production, biosynthesis, and physicochemical properties of these functional glycolipids will be necessary to fully exploit MELs in commercial applications.

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