Activation of Fibroblast and Papilla Cells by Glycolipid Biosurfactants, Mannosylerythritol Lipids.

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Abstract: Mannosylerythritol lipids (MELs), the extracellular glycolipids produced from feedstock by yeasts belonging to the genus Pseudozyma, are the most promising biosurfactants known due to its versatile interfacial and biochemical actions. In order to broaden the application in cosmetics, the cell activating property of MELs was investigated using cultured fibroblast and papilla cells, and a three-dimensional cultured human skin model. The di-acetylated MEL (MEL-A) produced from soybean oil significantly increased the viability of the fibroblast cells over 150% compared with that of control cells. On the other hand, no cell activation was observed by the treatment with MEL-A produced from olive oil. The mono-acetylated MEL (MEL-B) hardly increased the cell viability. The viability of the fibroblast cells decreased with the addition of more than 1 μg/L of MELs, whereas the cultured human skin cells showed high viability with 5 μg/L of MELs. Interestingly, the papilla cells were dramatically activated with 0.001 μg/L of MEL-A produced from soybean oil: the cell viability reached at 150% compared with that of control cells. Consequently, the present MEL-A produced from soybean oil should have a potential as a new hair growth agent stimulating the papilla cells.

Key words: cell activation, papilla cells, glycolipids, mannosylerythritol lipid.

1 INTRODUCTION

Mannosylerythritol lipids (MELs, Fig.1) are the most promising glycolipid biosurfactants produced from vegetable oils at a yield over 100 g/L¹ by yeast strains of the genus Pseudozyma. They are particularly interesting because they exhibit not only excellent surface-active properties, but also versatile biological activities². Moreover, MEL-A shows different biochemical actions including differentiation induction with respect to human leukemia³, rat pheochromocytoma⁴ and mouse melanoma⁵ cells, as well as high affinity binding towards different immunoglobulins⁶,⁷ and lectins⁸. We recently reported that MEL-A shows ceramide-like skin care⁹ and hair care¹⁰ properties, aiming to expand the commercial applications.

In the cosmetic industry, cell activation property is a crucial point of skin appendage morphogenesis. For instance, Minoxidil, which is the most commonly used drug for the treatment of androgenetic alopecia, has been reported to stimulate the proliferation of various skin and hair follicle cells¹¹. Among these cell types, the dermal papilla cells are known to induce follicle formation and hair growth by transdifferentiation of an adult epidermis¹². This means that the activation of the papilla cells is a key factor, considering the development of a new hair growth integrant. Based on our previous studies as indicated above, MELs are very likely to stimulate a variety of mammalian cells. We thus focused our attention on the cell activation properties of MELs toward cultured fibroblast and papilla cells.

Here, we report for the first time the activating property of MEL-A produced from soybean oil toward the cultured cells, and suggest the potential of MELs not only for a cost-effective moisturizer and/or hair care integrant but also for a new hair growth agent stimulating the papilla cells.
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purifi ed MEL-A and MEL-B fractions were then used in the

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2  EXPERIMENTAL

2.1 Production of mannosylerythritol lipids

Pseudozyma antarctica T-34 (as a MEL-A producer)¹³

and P. tsukubaensis NBRC 1940 (as a MEL-B producer)¹⁴

were cultured in a growth medium (4% (wt/vol) glucose,

0.3% NaNO₃, 0.03% MgSO₄, 0.03% KH₂PO₄, 0.1% yeast

extract, pH 6.0) at 25°C on a reciprocal shaker (150 strokes/

min) for 2 d. P. tsukubaensis NBRC 1940 was purchased

from National Institute of Technology and Evaluation of Ja-

pan. The obtained seed culture (0.1 mL) was transferred to

a 200-mL Erlenmeyer flask containing 20 mL of a produc-

tion medium (5% (wt/vol) olive oil or soybean oil, 0.3% 

NaNO₃, 0.03% MgSO₄, 0.03% KH₂PO₄, 0.1% yeast extract,

pH 6.0) at 25°C on a rotary shaker (250 rpm) for 7 d. The

produced MELs were extracted from the culture medium

with an equal amount of ethyl acetate.

2.2 Purification of MEL-A and MEL-B

The above organic layer was separated and evaporated.

The concentrated MELs were dissolved in chloroform and

then purified by silica-gel (Wako-gel C-200; Wako, Japan)

column chromatography. With a gradient elution of chloro-

form/acetone (10:0 to 0:10, vol/vol) mixtures as solvent sys-

tems¹⁵, purified MEL-A and MEL-B were obtained. The

purified MEL-A and MEL-B fractions were then used in the

following experiments.

2.3 Quantification of MEL-A and MEL-B by high-

performance liquid chromatography (HPLC)

The quantification of MEL-A and MEL-B were carried

out by HPLC on a silica gel column (Inertsil SIL 100A 5 μm,

4.6 × 250 mm; GL science, Japan) with a low temperature-

evaporative light scattering detector (ELSD-LT; Shimadzu,

Japan) using a gradient solvent program consisting of vari-

ous proportions of chloroform and methanol (from 100: 0 to

0: 100, vol/vol) at a flow rate of 1 mL/min¹⁴. The quantifica-

tion of MELs was performed based on the standard curve

using the pure MEL-A fraction as described previously¹⁴.

2.4 Structural analysis

The structure of the purified MEL-A and MEL-B was

confirmed by ¹H nuclear magnetic resonance (NMR) with a

Varian INOVA 400 (400 MHz) at 30°C using the CD₂OD

solution. The fatty acid profile of the MELs was analyzed by
gas chromatography-mass spectrometry (GC-MS) (Hewlett

Packard 6890 and 5973N) with a TC-WAX (GL-science, Ja-

pan) with the temperature programmed from 90°C (held for

3 min) to 240°C at 5°C/min, as described previously¹⁴.

2.5 Viability assay

The cell activation properties of MELs were evaluated

using cultured fibroblast and papilla cells, and a three-di-
mensional cultured human skin model, TESTSKIN²⁶ (Toyo-

bo, Japan). The fibroblast cells were cultured in a Dulbecco’
s Modified Eagle Medium (Gibco, USA) containing 10% of

fetal calf serum (FCS) for 24 h, and then the cells were

transferred into the Dulbecco’ s Modified Eagle Medium

containing 1% of FCS and different amounts of MELs for

48 h. The papilla cells were cultured in a Papilla Cell

Growth Medium (Toyobo, Japan) containing 10% of bovine

serum albumin for 24 h, and then the cells were trans-

ferred into the Dulbecco’ s Modified Eagle Medium con-

taining 1% of FCS and different amounts of MEL-A for 48 h.
The cells of TESTSKIN²⁶ were treated with a Assay Me-
dium (Toyobo, Japan) containing different amounts of

MEL-A for 48 h. The cell viability was colorimetrically
determined using a MTT assay kit (Nakalai, Japan). The cells

cultured in the medium without MELs were used as the

control.

3 RESULTS AND DISCUSSION

MEL-A and MEL-B were produced from olive oil [MEL-A

(O) and MEL-B (O)] and soybean oil [MEL-A (S) and MEL-B

(S)] as the sole carbon source by P. antarctica T-34 and P.

tsukubaensis NBRC 1940, respectively. The purified

MEL-A and MEL-B were obtained by the silica-gel column

chromatography and subjected to NMR analysis¹⁴. The

structures of MEL-A and MEL-B were confirmed as “di-

acylated and di-acylated 4-0-β-D-mannopyranosyl-meso-

erthritol” and “mono-acylated and di-acylated 1-O-β-D-
mannopyranosyl-meso-erethritol”, respectively. Based on

GC-MS analysis, the main fatty acids were C₈ and C₁₀ acids

in MEL-A, and the content of unsaturated fatty acids was

9.1% for MEL-A (O), 33.7% for MEL-B (O), respectively:

MEL-A (O) was composed of C₈ (38.7%), C₁₀ (43.2%),

C₁₂ (9.1%) , while MEL-B (S) was of C₈ (24.7%), C₁₀ (3.5%),

C₁₂ (21.7%), C₁₄ (24.5%), C₁₆ (5.7%). The main fatty

acids were C₈, C₁₂ and C₁₄ acids in MEL-B, and the

content of unsaturated fatty acids was 17.4% for MEL-B
Cell Activation Properties of Mannosylerythritol Lipids


Fig. 2 Activation of Cultured Cells by MEL-A and MEL-B.

The cultured fibroblast cells were incubated in a Dulbecco’s Modified Eagle Medium containing 10% of fetal calf serum (FCS) and MELs for 48 h, and the cell viability was estimated by MTT assay method: (A) the closed circle shows MEL-A (S) and opened circle is MEL-A (O), (B) the closed circle shows MEL-B (S) and opened circle is MEL-B (O). Vertical bars show the standard error of the mean based on three independent measurements.

(0), 43.9% for MEL-B (S), respectively; MEL-B (O) was composed of C_{10} (46.8%), C_{12} (16.1%), C_{12} (9.7%), C_{14} (18.7%), C_{14} (7.7%), and MEL-B (S) was of C_{9} (23.6%), C_{9} (1.6%), C_{12} (11.6%), C_{12} (12.5%), C_{14} (1.9%), C_{14} (8.3%), C_{14} (21.5%). Therefore, the MELs produced from olive oil composed of the highly saturated fatty acids compared with that from soybean oil as described previously [16].

These two purified MELs were then used in the following experiments.

To estimate the cell activation properties of MELs, the cultured fibroblast cells were incubated in the medium containing different amounts of MELs for 48 h, and then the viability was measured by MTT assay method (Fig. 2). The cell viability increased up to 170% by the treatment with 0.001 μg/mL MEL-A (S), whereas MEL-A (O) showed no positive effect. Increase of the MEL-A (S) concentration resulted in the decreased of the cell viability. The observed negative effect may be due to the peeling of the cells from the culture plate, considering that MEL-A shows high surface activity. Both of MEL-B (S) and MEL-B (O) hardly increased the viability of the fibroblast cells. Accordingly, MEL-A (S) showed the highest effect among MELs tested, suggesting that the fatty acid composition of MEL should be critical for the cell activation property.

To elucidate the observed negative effect of MEL-A (S) at high concentrations, we further checked the activation property using the cultured human skin model, in which fibroblasts and epidermal keratinocytes were fixed on the membrane. After treatment with 5 and 50 μg/mL of MEL-A (S), the viability of the cultured human skin cells significantly increased (Fig. 3). Accordingly, MEL-A (S) was confirmed to show no negative effect even at high concentrations below 50 μg/mL, and thus the glycolipid should have a potential for the activation of human skin cells.

Fig. 3 The Effect of MEL-A (S) on Three-dimensional Cultured Skin Model.

The cells of the three-dimensional cultured human skin model were treated with 5 μg/mL or 50 μg/mL of MEL-A (S). The cells viability was estimated by MTT assay method. The 0.5% of ethanol was used as the control solution. Vertical bars show the standard error of the mean based on three independent measurements. * p < 0.05, significantly different when compared with the control value, lauryl glucoside (Student’s t-test).
Fig. 4 The Effect of MEL-A (S) on the Cultured Papilla Cells.

The cultured papilla cells were incubated in a Papilla Cell Growth Medium containing 10% of bovine serum albumin and MEL-A (S) for 48 h, and the cells viability was estimated by MTT assay method. Vertical bars show the standard error of the mean based on four independent measurements. *p < 0.05, significantly different when compared with the control value, lauryl glucoside (Student’s t-test).

We also investigated the activation of the papilla cells by MEL-A (S). The cultured papilla cells were incubated in the medium containing different amounts of MEL-A (S) for 48 h, and then the viability was measured by MTT assay method. Interestingly, MEL-A (S) clearly increased the cell viability over 150% compared with that of control (Fig. 4). Increase of the MEL-A (S) concentration resulted in the decreased of the viability as observed above. The papilla cell, which is composed of a specialized fibroblast exited in the base of the follicle, is reported to control the number of matrix cells and the size of hair [17]. The topical administration of MEL-A (S) into mice showed significant stimulation of the hair growth [18]. From these lines of evidence, MEL-A (S) is likely to have a great potential as a new drug for the treatment of alopecia.

References
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