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"Development of a Bioemulsion from Novel Skin Microbiome EPI-7i: A Green Science Approach to Cosmetic Formulation"

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1. Introduction

Traditional cosmetic formulations often involve complex chemical processes and significant energy consumption, primarily relying on synthetic surfactants to emulsify hydrophilic and lipophilic components [1,2]. These surfactants, primarily synthesized from petroleum, demonstrate resistance to biodegradation, and pose significant environmental hazards, while also contributing to undesirable textures and potential dermal irritation [3,4]. To address these limitations, microbial biosurfactants are emerging as viable alternatives [5-8].

Our research group identified a novel actinobacteria species, *Epidermidibacterium keratini* EPI-7, uniquely exists in the group of 20s healthy skin [9-11]. In our previous studies, we highlighted that the *E. keratini* EPI-7 derived postbiotics significantly enhanced skin barrier function, elasticity, and dermal density. Notably, the treatment with the EPI-7 ferment filtrate led to an increased abundance of commensal microbes on the skin surface, underscoring its positive impact on the skin microbiome [10,11].

As we delved deeper into the bioavailability and physiological properties of *E. keratini* EPI-7, a question arose: how does *E. keratini* EPI-7 survive on human skin? Tchoupa *et al*. mentioned that the abundance of free fatty acids in epidermal lipid

lamellar membranes is an attractive source of nutrients for skin microbiome [12]. However, free fatty acids must be mobilized from the rather inaccessible lamellar-membrane compartments, prompting skin bacteria to secrete surfactant molecules that solubilize free fatty acids [13-15]. In our previous studies, it was confirmed that *E. keratini* EPI-7 expressed the lipid degrading enzymes such as esterase (C4) and esterase lipase (C8), producing various types of phospholipids in the metabolites [9-11]. Building on this foundation, we hypothesize that *E. keratini* EPI-7 possesses the capability to metabolize sebum and other types of lipids such as natural carrier oils.

Unlike traditional approaches that utilize skin microbiomes as active ingredients, our research expanded the application to cosmetic formulation technology via the metabolism of *E. keratini* EPI-7. We hypothesized that *E. keratini* EPI-7 could metabolize natural carrier oils, given its environment. Through gradual adaptation involving natural oils, we developed an improved strain, *E. keratini* mutant EPI-7 (EPI-7i), capable of bioconverting triglycerides into an emulsion-like phase.

In this study, we introduced a biotechnology-derived cosmetic formulation platform utilizing a novel skin microbiome, *E. keratini* mutant EPI-7 (EPI-7i), focusing on the conversion of triglycerides from the natural carrier oils. The bioconversion of natural oil by *E. keratini* EPI-7, referred to as bioemulsion, features a light, creamy, and hydrating texture. This bioemulsion was analyzed to identify key emulsifying molecules, and its bioavailability was evaluated through *in-vitro*, *ex-vivo*, and clinical tests. These assessments aim to determine its potential as a feasible option for green science-based cosmetic formulations.

2. Materials and Methods

2.1. Materials

E. keratini EPI-7i was provided by our research group. Macadamia seed oil was obtained from Floratech (Arizona, USA). The lipid standards were obtained from Avanti Polar Lipids, Inc. (Alabaster, USA). The other chemical reagents were prepared based on the previously described methods [16].

2.2. Synthesis of *E. keratini* EPI-7i lipid metabolites

2.2.1. Seed Culture and Sub-Culture Preparation

A medium was composed of pancreatic digest of casein (17.0 g/L), papain digest of soybean (3.0 g/L), dextrose (2.5 g/L), NaCl (5.0 g/L), and K_2HPO_4 (2.5 g/L) in 1 L of distilled water. Cultivation occurred at 30°C aerobically. 10 mL was inoculated in a 100 mL baffled flask and diluted to 10% with sterile saline. To prepare sub-

culture preparation, the medium contained papain digest soybean (0.1 g/L), yeast extract (1.0 g/L), glycerin (1.0 g/L), KH_2PO_4 (4.5 g/L), K_2HPO_4 (3.0 g/L), and macadamia seed oil (50 mL/L) in 1 L of distilled water. 100 mL was used per 500 mL flask, with seed culture at 100 g/L, maintained aerated at 30°C.

2.2.2. Preparation of BE10

The medium consisted of papain digest soybean (0.1 g/L), yeast extract (1.0 g/L), glycerin (0.1 g/L), KH_2PO_4 (4.5 g/L), K_2HPO_4 (3.0 g/L), macadamia seed oil (200 mL/L), and others in 1 L of water, loaded in a 5 L bioreactor with 3 L total volume. Inoculum was added at 100 g/L under conditions of 30°C, 3 NL/min airflow, and 600 rpm agitation for 5 days of incubation. Following the completion of fermentation, the culture broth was subjected to centrifugation at 8,000 rpm for 30 minutes. The supernatant was carefully collected, while the cell debris was discarded. To remove residual microorganisms and other impurities, the recovered supernatant was processed using microfiltration (Vivaflow 50R, Sartorius) with dilution in half to adjust the content of bio-converted macadamia seed oil by *E. keratini* EPI-7i (INCI: Epiderimdibacterium keratini/macadamia ternifolia seed oil ferment). The bioemulsion containing 10% of bio-converted macadamia seed oil by *E. keratini* EPI-7i (BE10) was analyzed for the further study.

For the comparison, the control emulsion was prepared with emulsification of 10% of macadamia seed oil with synthetic surfactants including acrylates/C10-30 alkyl acrylate crosspolymer, polysorbate 60, glyceryl stearate, PEG-100, stearate, tromethamine.

2.3. Analysis

2.3.1. Stability and Lipid Analysis

To evaluate the stability of BE10, the particle size and zeta potential were measured using dynamic light scattering, SZ-100 Horiba. The lipid components of *E. keratini* EPI-7i lipid metabolites were detected with using UPLC-MS/MS analysis. The sample was injected to Ultimate 3000 UHPLC system (Thermo Fisher Scientific, USA) with reverse-phase ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm) (Waters, USA). Mass spectrometric analysis was carried out on a Triple TOF 5600+ System (AB SCIEX, USA).

2.4. Efficacy Evaluation

2.4.1. *In-vitro* and *Ex-vivo* Skin Performance Evaluation

For *in-vitro* experiments, 3D reconstructed human full skin model (Keraskin-FT™) and culture media were purchased from Biosolution Co., Ltd. (Seoul, Korea). In case of *ex-vivo* experiments, human skin explant tissues were provided after surgical removal of abdominal skin tissues of donor patients. Adipose tissue attached to skin explant was removed, punched into 8 mm size tissues and transferred to trans-well plate for culture. Skin explant tissues were stabilized for 24 hours under 37°C, 5% CO_2, humidified culture condition. To investigate efficacy of BE10 on the skin, BE10 was treated topologically on 3D skin model and also on UVB irradiated to inspect

possible skin protecting effects. UVB irradiation was applied with 500 mJ/cm^2 intensity. After 72 hours of BE10 treatment, relative mRNA expression levels were analyzed by real-time PCR, protein expressions and structural differences were analyzed using human skin explant tissues by immunohistochemistry and Masson-Trichrome collagen staining assay.

2.4.2. RNA Sequencing

Analysis of differentially expressed gene was performed by ILLUMINA instrument. We utilized 3D full skin model containing epidermal and dermal layer, analyzed gene alteration in whole genome level.

2.4.3. Clinical Study

A total of 21 study subjects, aged 30 to 60 years, were enrolled in this study. All 21 subjects (mean age 47.10±9.18 years, all female) completed the study in accordance with the study protocol. The subjects were instructed to apply appropriate amount of the control and BE10 on different side of the face twice daily for 4 weeks to evaluate its skin performance. All subjects provided written informed consent.

3. Results

The skin's surface contains various lipids, including triglycerides, which the skin microbiome uses as nutrients for a symbiotic relationship with the skin. Figure 1 illustrates the formulation process for a novel skin microbiome-based cosmetic emulsion. Leveraging the ability of *E. keratini* EPI-7 to metabolize skin lipids and produce beneficial active ingredients, we developed the improved strain *E. keratini* EPI-7i. This strain can metabolize a range of natural oils, such as olive, sunflower seed, and rapeseed oils, and notably exhibits high odor stability over the storage time period in the case of bio-converted macadamia seed oil. Our trials indicated that *E. keratini* EPI-7i can bio-convert up to 30% of macadamia seed oil into an emulsion-like phase, referred to as bioemulsion.

The bioemulsion was formed through bioconversion using *E. keratini* EPI-7i, and its stability was evaluated using various parameters. Figure 2 (a) illustrates the transformation of two separate phases—macadamia seed oil and the culture medium—into a homogeneous emulsion-like phase following the bioconversion process. Optical microscopy confirmed the presence of emulsion particles. For further analyses, the bioemulsion containing 10% of *E. keratini* EPI-7i lipid metabolites (BE10) was selected. Figures 2 (b) and (c) depict the formation and stability of BE10. The transmission electron microscopy (TEM) images in Figure 2 (b) reveal spherical emulsion droplets exhibiting a bluish translucent color, indicative of a nanoemulsion as a result of the Tyndall effect [17]. Figure 2 (c) presents the stability of BE10 under various storage conditions (4°C, 25°C, 37°C, 45°C) and sunlight exposure over 0, 7, 30, and 60 days.

At Day 0, the average particle size of nanoemulsion was approximately 108.3 nm, and by Day 60, the particle size remained similar or smaller than that at Day 0, indicating high stability.

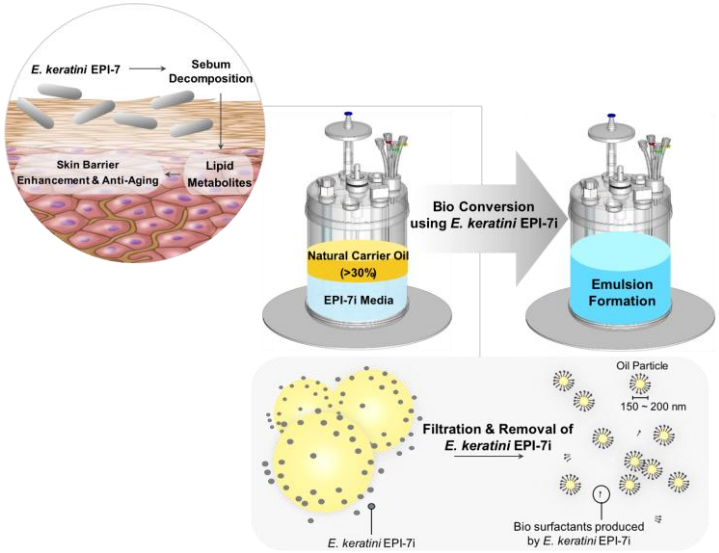

Figure 1. Schematic diagram of production of emulsion formulation through bio conversion of natural oil using *E. keratini* EPI-7i.

To further assess the stability of BE10, we measured turbidity, viscosity, and zeta potential at room temperature over 0, 7, and 60 days. Table 1 illustrates that dispersing 0.5% BE10 in distilled water resulted in a 2.3% change in optical density at 600 nm after 60 days, while viscosity showed a variation of 2.2% over this period. Notably, the zeta potential increased from Day 0 to Day 60, indicating that BE10 maintains stability over time. These findings suggest that the bioemulsion not only retains its physical properties but also exhibits improved stability characteristics.

The lipid composition of *E. keratini* EPI-7i metabolites produced through the bioconversion of macadamia oil was analyzed using TOF-MS/MS, revealing a diverse range of phospholipids. Notable identifications included phosphatidylglycerol (PG) species such as PG 32:1, PG 33:1, and PG 34:1, as well as phosphatidylinositol 34:0. The metabolites also contained various lysophospholipids, including lysophosphatidylethanolamine (LPE) 14:0 and LPE 16:0, alongside lysophosphatidylinositol (LPI) 16:0 and LPI 14:0. The formation of O/W emulsion and the stability of BE10 is attributed to the production of these phospholipid variants, contributing to its high stability.

We investigated the efficacy of BE10 with results presented in Figure 3. The gene expression and histological changes following BE10 treatment on 3D skin and skin explant tissues showed significant improvements compared to an emulsion made with synthetic surfactant.

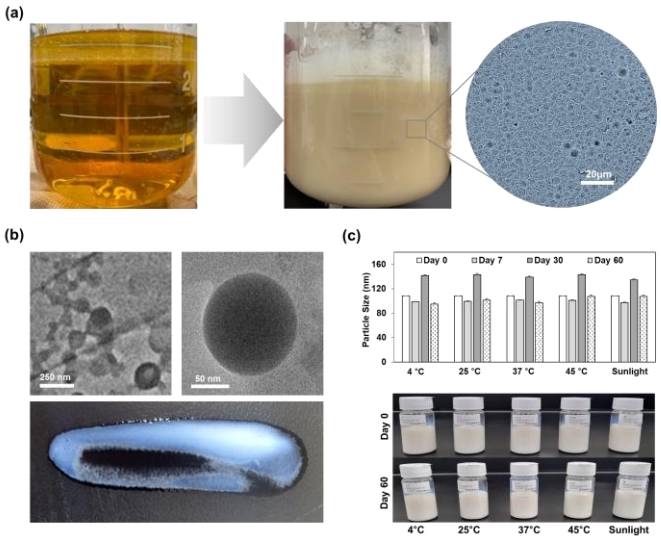

Figure 2. Characteristics and stability of bioemulsion. (a) Images of macadamia seed oil with *E. keratini* EPI-7i before (left) and after (right) bioconversion, along with optical microscopy of emulsion particles; (b) TEM image of BE10 showing emulsion droplets (above) and spread of BE10 (below); (c) Stability assessments of BE10 under various storage conditions (4°C, 25°C, 37°C) and sunlight over 0, 7, 30, and 60 days (above) with corresponding images (below).

Table 1. Evaluation of the stability of BE10.

Condition	Day 0	Day 7	Day 60
OD_{600nm}	0.87	0.84	0.85
Viscosity (Cp)	4400	4280	4320
Zeta Potential (mV)	-94	-108.3	-127.3

*OD_{600nm}: 0.5% of BE10 in distilled water solution was used to measure the turbidity.

Specifically, Figure 3 (a) indicates enhanced expression of barrier-related genes such as claudin-1 and ceramide synthase 3, which are crucial for skin protection. Figures 3 (b) and (c) demonstrate that BE10 effectively improved indicators of photo-damaged skin, such as skin elasticity (elastin, fibrillin) and hydration factors (aquaporin, hyaluronan synthase), which were diminished under UVB exposure. Histological analysis, depicted in Figure 3 (d), further corroborated the positive effects of BE10, particularly in the restoration of filaggrin and collagen levels. Overall, BE10 enhanced skin performance by reinforcing the skin barrier and improving moisture and elasticity, particularly in models of photo-aging. In contrast to conventional synthetic surfactant emulsions, BE10 showed superior efficacy, suggesting that the bioconversion process of E. keratini EPI-7i provides eco-friendly benefits and positive bioactivity for the skin without the need for additional active ingredients.

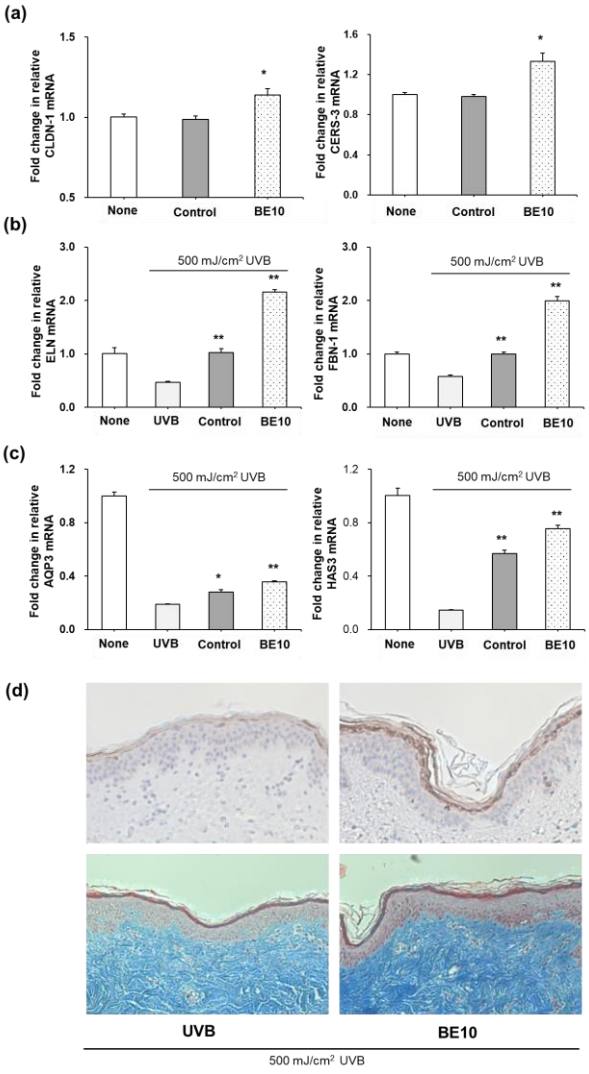

Figure 3. Gene expression and histology analysis of BE10-treated skin explants. (a) Relative mRNA expression of claudin-1 (CLDN-1) and ceramide synthase-3 (CERS-3), indicating improved skin barrier function; (b) Increased mRNA levels of elastin (ELN), and fibrillin (FBN-1), indicating anti-aging effects; (c) Upregulated expression of aquaporin-3 (AQP3) and hyaluronic synthase 3 (HAS3), demonstrating moisturizing properties; (d) Immunohistochemistry results: filaggrin stained red (above) and collagen stained blue via Masson's Trichrome (below).

RNA sequencing analysis of BE10 treated 3D skin model also correlated to the previous efficacy results. Expression levels of differentially expressed genes were analyzed, grouped as functionally related pathway. Sequencing results shown in Figure 4 indicated that the most up-regulated pathway in BE10 treated group was keratinocyte differentiation, which correlates with skin barrier enhancing results of Figure 3.

A clinical study involving 21 participants aged 30 to 60 assessed the effects of BE10 on skin parameters such as elasticity, moisture content, wrinkle reduction, itch relief, and hyperpigmentation, shown in Figure 5.

Facial moisture in the BE10 group rose from 63.42 ± 9.42 to 67.06 ± 9.77 (5.74% increase), whereas the control group experienced a slight decrease, shown in Figure 5 (a).

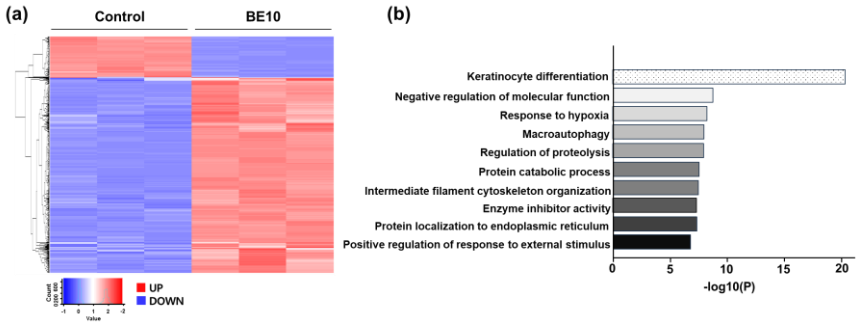

Figure 4. Differentially expressed gene analysis with the treatment of BE10 and control on the 3D skin models. (a) The up and down regulations of genes with n=3 for each sample; (b) The top 10 up-regulated gene upon the treatment of BE10 on the 3D skin.

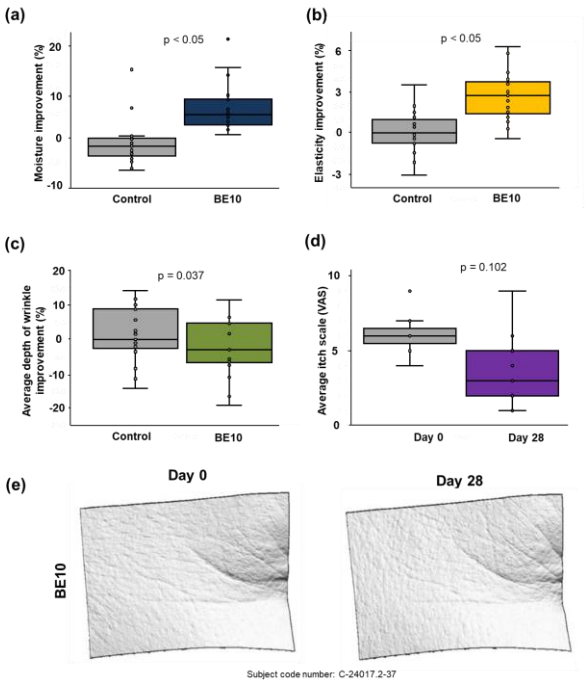

Figure 5. The clinical tests with the treatment of BE10 for 4 weeks. (a) The moisture improvement; (b) The elasticity improvement; (c) The average depth of wrinkle improvement; (d) The itchiness improvement; (e)The decrease of wrinkle formation.

Figure 5 (b) showed a significant increase in skin elasticity from 69.98% ± 3.13 to 71.84% ± 3.16 (2.66% improvement), while the control group's change was minimal (0.06%). In terms of wrinkle reduction, shown in Figure 5 (c), the BE10-treated group decreased wrinkle depth from 65.52 μm ± 18.05 to 63.24 μm ± 16.52 (3.48% reduction), in contrast to a 1.67%

increase observed in the control group. Additionally, Figure 5 (d) demonstrated that the BE10 group reported a 42.48% reduction in itchiness due to dryness, while the control group saw a decrease of 33.61. Overall, these results indicate that BE10 significantly enhances skin health, outperforming the control in elasticity, hydration, wrinkle reduction, and itch relief.

4. Discussion

In this study, we discovered that specific skin microbiome can metabolize natural oils to produce the biosurfactants. This finding highlights the potential to leverage the metabolic capabilities of skin microbiomes not only as cosmetic active ingredients but also as formulation technologies. Building upon this discovery, we explored the potential of *E. keratini* EPI-7i in creating a stable bioemulsion via the bioconversion of natural oils, specifically macadamia seed oil. The bioconversion process transformed the oil and medium into a homogeneous emulsion, designed as BE10, with its stability validated through comprehensive analyses, including TEM and optical microscopy. This stability is largely credited to a diverse range of phospholipids generated during the process, as elucidated by TOF-MS/MS analysis. The resulting bioemulsion exhibited considerable enhancements in skin health parameters compared to the synthetic formulation. These included improved barrier function, hydration, and elasticity. Clinical trials confirmed these benefits by showing significant improvements in skin conditions such as elasticity and moisture, alongside a reduction in wrinkles and hyperpigmentation. Overall, these findings suggest that *E. keratini* EPI-7i bioemulsions could serve as a sustainable and effective alternative in skincare products, offering enhanced bioactivity. Future research may further uncover their long-term benefits and broader applications in skincare.

5. Conclusion

The skin microbiome is pivotal in maintaining homeostasis and has significantly influenced the evolutionary trajectory of human skin. Our research elucidates that these microbial communities metabolize sebum to generate amphiphilic molecules, which are crucial for preserving the skin's equilibrium. This study presents compelling evidence supporting the utilization of the novel microbiome strain *E. keratini* Epi-7i in developing sustainable and efficacious bioemulsions for cosmetic applications. Furthermore, our findings indicates the possibility that nanoemulsions are likely being generated via the lipid metabolic activities of the skin microbiome, subtly affecting human skin homeostasis. This underscores the necessity for continued research into this profound interplay.

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