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A Trial for the Construction of a Cosmetic Pattern Map Considering Their Effects on Skin Microbiota—Principal Component Analysis of the Effects on Short-Chain Fatty Acid Production by Skin Microbiota *Staphylococcus epidermidis*

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Abstract: Cosmetics in the world have various characteristics, but differences in their effects on skin-domesticated bacteria were not known. In this study, to obtain insights into their effect on skin microbiota, we established an anaerobic culture and short-chain fatty acid measurement system using *Staphylococcus epidermidis*, a representative bacterium of indigenous skin bacteria. This system was used to extract the characteristics of nine cosmetics and classify them. Cosmetics containing ferments of rice and soy were clustered in the vicinity, although one cosmetic containing soy ferment was clustered distantly from other similar cosmetics. Cosmetics from South Asia and those containing natural plant extracts were clustered in the vicinity. This trial could be utilized as a pattern map of cosmetics in terms of their effects on skin microbiota.

Keywords: cosmetics; skin microbiota; *Staphylococcus epidermidis*; lactic acid; PCA

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1. Introduction

Cosmetics are applied to the skin for the purpose of enhancing beauty. Before the establishment of the modern chemical industry, there were few concepts of makeup and skincare, and most cosmetics used plant-derived ingredients or minerals. In Japan, makeup products such as white powder, safflower, and eyebrow ink, and plant-derived skincare products such as loofah water and flower dew [1,2] began to become popular among the general public during the Edo period (AD 1600–1868). The establishment of the chemical industry in the 18th and early 19th centuries led to the use of glycerin as a moisturizing ingredient and the spread of cosmetics made with chemical products. For this reason, the cosmetics industry established in the 20th century, combined with the petrochemical industry established in the early 20th century, almost exclusively used petroleum-derived chemical products. However, such chemical products have not been applied on human skin in the past, and due to the problem of petroleum-derived trace constituents, they have been causing various health problems since the 1970s. For example, skin diseases such as melanosis [3] and chemical sensitivity have been reported. As a result, from the 1980s, many cosmetics using naturally derived ingredients began to appear on the market. Furthermore, taking advantage of traditional Japanese fermentation technology, many cosmetics containing fermented products were developed in Japan [4,5]. However, there have been no studies to date that have revealed the effects of cosmetics containing such natural or fermentation-derived ingredients on skin bacteria, let alone cosmetics made with chemical products. There are cosmetics from various cultural backgrounds in the world, and they have completely different characteristics. However, with the development

of logistics, cosmetics from all over the world are now available, and there have been no studies comparing the effects of these cosmetics on skin microbiota.

The properties of cosmetics vary depending on the cultures of countries. For example, in Japan, many cosmetics are based on fermented products because of the country's long tradition of fermented foods. On the other hand, in South Asia, cosmetics based on medicinal herbs are considered common due to the Ayurvedic tradition of using medicinal herbs. In Europe, where the cosmetics industry first emerged, many cosmetics are chemical-based, and many are dairy- and plant-based due to the traditions of dairy farming and Unani medicine. However, there are no studies that report such regional characteristics because there is no system to identify the properties of cosmetics. Therefore, we considered that analyzing cosmetics from these areas might generate some information on the cultures of respective cosmetics.

Skin is said to be "the largest organ of the human body" [6] since it covers our entire body, occupying a total area of about 1.6 square meters (for an adult) and weighing about 15–16% of our body weight. Therefore, it is believed that maintaining healthy skin leads to the maintenance of the overall health of the body. Human skin is inhabited by about 1000 species of bacteria, which are referred to as dermal commensal bacteria. The skin commensal flora is said to be the second largest flora in the human body after the intestinal flora [7]. They are classified into three categories according to their function on the host: beneficial bacteria, opportunistic bacteria that change their role depending on the balance between beneficial and harmful bacteria on the skin, and harmful bacteria that cause skin problems. Among these, *Staphylococcus epidermidis*, which is the most ubiquitous bacterium on human skin and is known as the representative of good bacteria, degrades sweat and sebum on the skin and produces glycerin and fatty acids. The glycerin produced by these indigenous skin bacteria strengthens the skin barrier function [8], and the fatty acids keep the pH level of the epidermis acidic and inhibit the growth of bad bacteria [9]. Other short-chain fatty acids derived from indigenous skin bacteria have also been reported to have various effects on the skin. For example, lactic acid has been reported to have a peeling effect on the skin and a softening effect on the stratum corneum [10]. In addition, it has recently been reported that lactate produced by indigenous skin bacteria [11] and *Staphylococcus aureus* act on the immune response of the host via the production of IL-10 and control skin pathogens [12]. Succinic acid inhibits the growth of *Propionibacterium acnes*, a causative agent of acne [13], and propionic acid inhibits the growth of *Staphylococcus aureus*, a representative of hazardous bacteria [14]. On the other hand, acetic acid and formic acid are known to be highly irritating to the skin [15]. Therefore, although there might be other factors such as temperature, environment, pollution, and disequilibrium of microbiota in vivo, insight into the effects of cosmetics on short-chain fatty acids produced by *S. epidermidis* in vitro might provide some information on the effects of cosmetics on skin health in vivo.

In this study, we attempted to establish a cosmetics pattern map that can extract and compare the characteristics of cosmetics based on the short-chain fatty acids produced by *S. epidermidis*.

2. Materials and Methods

2.1. Materials

The bacteria used were *Staphylococcus epidermidis* (NBRC100911) purchased from the Bioligical Resource Center, NITE (NBRC, Kisarazu, Japan).

The nine cosmetics used in this study and the main ingredients used in the cosmetics are listed below (Table 1). Since we focused on the properties of fermented cosmetics, we picked up several Japanese fermented cosmetics. As a reference, we picked up a product from South Asia, where Ayurvedic culture is dominant.

Table 1. Cosmetics used in this study.

Name	Cosmetics Containing Soy Milk Fermentation Liquid	Cosmetics Containing Soy Milk Fermentation Liquid	Lotion Containing Rice Ferment	Cosmetics Containing Rice Ferment	Cosmetics Containing Plant Extract	Cosmetics Claiming Plant Extract	Cosmetics Claiming Natural Ingredients	Cosmetics Containing Loofah Water	Preservative Cosmetics
Region	Japan	Japan	Japan	Japan	Japan	South Asia	Japan	Japan	Japan
Code	Soy-ferment A	Soy-ferment B	Rice-ferment A	Rice-ferment B	Plant	South Asia	Natural	Loofah	Preservative
Ingredients	Water, glycerol, 1,3-butylglycol, ethanol, isoflavone, fermented soy	Water, glycerol, dipropylenegly-col, ethanol, fermented soy	Water, glycerol, butylene glycol, fermented rice, glutamic acid	Kojic acid, allantoin, algae extract, plant extract, fermented soy	Water, butylene glycol, ethanol, glycerol, allantoin, plant extract	Water, flavor, plant oil, propylene glycol, phenoxyethanol	Water, butylene glycol, glycerol, pentylene glycol, betaine, acryloyloxyethyl phosphoryl choline polymers, fermented extract	Loofah extract	Vitamin C derivative, ethoxydiglycol, plant seed extract, butylene glycol, disodium edetate

2.2. Reagents

The following culture media and reagents were used to culture the bacteria.

Bacterial pre-culture: 702 medium (High polypeptone [Wako Pure Chemical Industries, Ltd., Osaka, Japan], yeast extract [Wako Pure Chemical Industries, Ltd., Osaka, Japan], magnesium sulfate heptahydrate $MgSO_4$-$7H_2O$ [Nakalai Tesque Co., Kyoto, Japan]). After preparation of the medium, pH was adjusted to 7.0. Co-culture of bacteria and cosmetics: GAM medium (Modified GAM Bouillon [Nissui Pharmaceutical Co., Tokyo, Japan]).

2.3. Nitrogen Replacement in the Culture Vessel

A 10 mL headspace vial (vial, 45 mm × 22 mm, Agilent technologies Inc., Santa Clara, CA, USA) was used as the culture vessel. Nitrogen was supplied from the connecting tube of a nitrogen gas cylinder to the vials using silicone tubes (9 × 12 mm); 1000–5000 μL Pipet Tip. GAM medium was added to 5 mL vials. A total of 5 μL of resazurin diluent (1 mg/mL), a redox indicator, was added at the time of GAM medium preparation, and the color of the medium changed from reddish purple to yellow derived from the medium as a discrimination of anaerobic condition. The tip of the nitrogen inflow device was inserted into vials. The entire area was covered with aluminum foil, and holes were drilled for oxygen exit. An anaerobic environment was created by flowing nitrogen gas and bubbling for 5 min. After gas displacement, a crimp cap (septum type: molded PTFE/butyl, Agilent technologies Inc., USA) was immediately set on the vial and immediately sealed using a hand crimper (G.L. Science Corporation, Tokyo, Japan). The culture medium was then sterilized via autoclaving (121 °C, 15 min).

2.4. Co-Culture of S. epidermidis and Cosmetics

S. epidermidis was inoculated into 5 mL of 702 medium and pre-cultured at 37 °C for 24 h. In the main culture, the pre-culture solution was added to the GAM medium prepared in the method to achieve an initial bacterial concentration of 1.0×10^7 cells/5 mL. A microsyringe (Ito Manufacturing Co., Ltd., Nagano, Japan) was used for the addition. A total of 0.25 mL (5% of the total volume of 5 mL) of the cosmetic product to be studied was added and incubated at 37 °C for 24 and 48 h. A total of 6 samples per cosmetic product were prepared: vehicle-added control group (0, 24, 48 h) and cosmetic product-added group (0, 24, 48 h).

2.5. Extraction of Exogenous Metabolites (Removal of Foreign Substances)

After co-culturing as described above, the culture medium was centrifuged (24,000 rpm, 5 min), and 2.0 mL of the supernatant was collected as exogenous metabolites. Further centrifugation (14,000 rpm, 1 min) was performed to ensure removal of bacteria, and 1.5 mL of the supernatant was removed from remnants using a 0.45 μm membrane filter (Shimadzu GLC Corporation, Kyoto, Japan). A total of 1.0 mL was added to a vial for HPLC, covered with a lid, and set in an autosampler for HPLC (L-2200, Hitachi, Ltd., Tokyo, Japan). Randomization was performed using the random function in Microsoft Excel for the analysis, and all analyses of each cosmetic were performed on the same day.

2.6. Short-Chain Fatty Acid (BTB Method) Analysis via High-Performance Liquid Chromatography (HPLC)

Analysis via HPLC was performed using an RSpak KC-811 column (particle size 6.0 μm, inner diameter 8 mm, length 300 mm, G.L. Science Corporation), column oven: 40 °C, flow rate: 0.5 mL/min (A), 0.6 mL/min (D), buffer A: 3 mM $HclO_4$, buffer D: 0.1 mM BTB and 15 mM Na_2HPO_4, analysis time: 80 min.

2.7. Peak Identification

Peak identification was based on the retention time in the chromatogram. The obtained area value data for short-chain fatty acids were converted to an Excel file and used for subsequent statistical analysis.

2.8. Statistical Analysis

SIMCA-P+ ver. 13.0 (Umetrics, Umeå, Sweden) was used for principal component analysis.

3. Results

3.1. Establishment of an Anaerobic Culture and Short-Chain Fatty Acid Production Measurement System for S. epidermidis

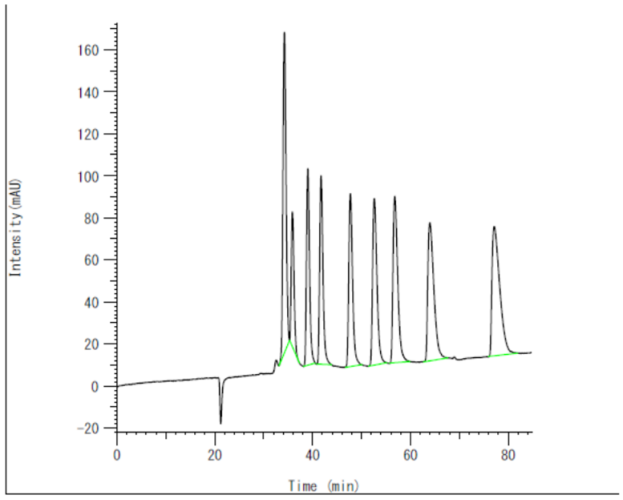
First, to investigate whether metabolites of *S. epidermidis*, an indigenous skin bacterium, can be quantitatively detected in an anaerobic environment that mimics skin pores, media with or without *S. epidermidis* cultured anaerobically for 0, 24, and 48 h were subjected to HPLC for analysis. As a result, six peaks were detected and identified as six short-chain fatty acids (succinic acid, lactic acid, formic acid, acetic acid, propionic acid, and isobutyric acid). This suggested that *S. epidermidis* metabolites could be quantitatively detected under these culture conditions. The concentrations (μg/mL) of the short-chain fatty acids were calculated from the detected peak area values, and calibration curves for each short-chain fatty acid. Using these values, we compared the amount of short-chain fatty acid production by incubation time and found that lactic acid and acetic acid increased in the 24 and 48 h incubation samples (Figure 1). From this result, we considered that we could construct a system to simulate the metabolism of indigenous skin bacteria in skin pores.

3.2. Change in Short-Chain Fatty Acid Production via S. epidermidis upon Addition of Cosmetics

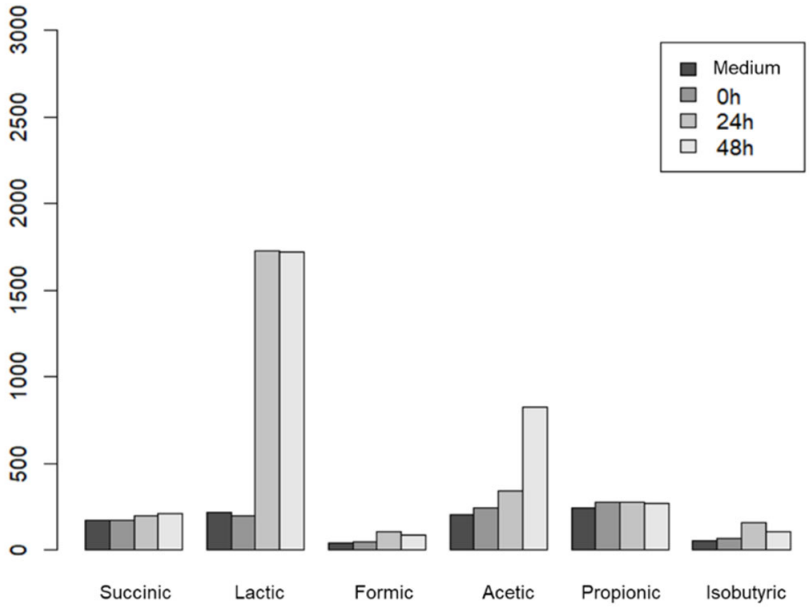
Since we were able to construct a system for analyzing metabolites of *S. epidermidis*, as described above, we decided to examine whether the metabolites changed when actual cosmetics were added to the culture. *S. epidermidis*, alone cultured for 0, 24, and 48 h, and *S. epidermidis* added with various cosmetics for 0, 24, and 48 h were subjected to HPLC and analyzed. The results showed that there were various patterns depending on the cosmetics (Table 2), which may allow us to distinguish and clarify the effects of cosmetics on indigenous skin bacteria.

3.3. Classification of Cosmetics Based on Principal Component Analysis of the Amount of Short-Chain Fatty Acids Produced by Indigenous Skin Bacteria

In order to gain a comprehensive insight into the pattern of short-chain fatty acid production by each cosmetic product obtained above, a principal component analysis was conducted using the values as explanatory variables. As a result, an axis separating lactic acid and other short-chain fatty acids as the first principal component was obtained, along with an axis separating succinic acid, formic acid, and isobutyric acid as the second principal component (Figure 2). The contribution of the first and second principal components (PC1 and PC2) was 44.8% and 24.7%, respectively. In the score plot, the samples were divided into two groups. The first group was the groups of rice-fermented cosmetics and soy-fermented cosmetics, and the second group was South Asian cosmetics, plant extract cosmetics, and natural cosmetics. One cosmetic containing soy ferment was clustered in the vicinity of the preservative. This finding suggested that it would be possible to classify cosmetics according to their characteristics and to identify the substances that contribute to this classification.



(**A**)



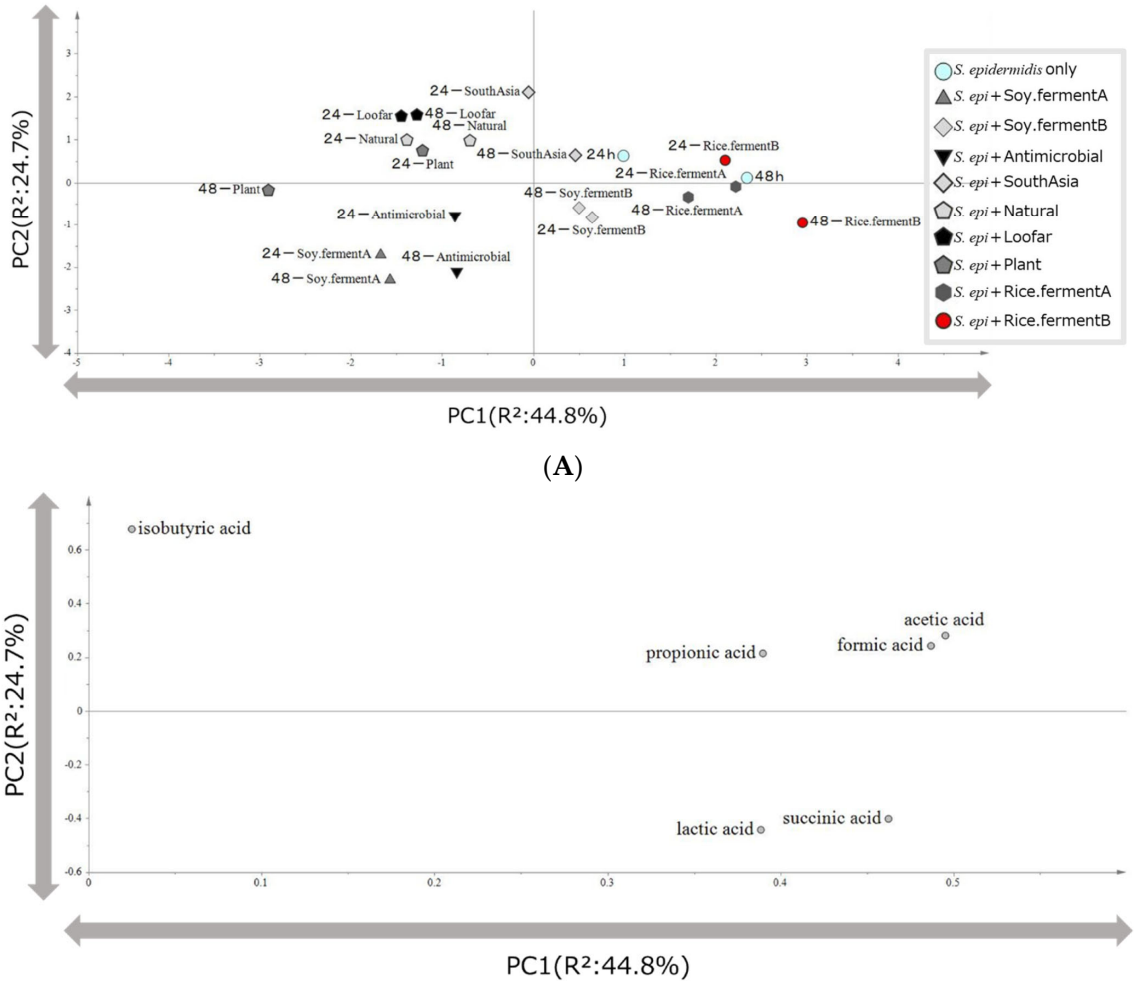
(**B**)

Figure 1. Short-chain fatty acid concentration of the culture of *S. epidermidis*. (**A**) A representative chromatogram. (**B**) Concentrations of short-chain fatty acids after incubation for 24 or 48 h. *S. epidermidis* was cultured in nitrogen-purged GAM medium anaerobically. Concentrations of cultures incubated with *S. epidermidis* for 0, 24, and 48 h were analyzed using HPLC. Retention time; Succinic acid: 34.3 min, lactic acid: 35.9 min, formic acid: 39.0 min, acetic acid: 41.8 min, propionic acid: 48.0 min, isobutyric acid: 57.4 min.

Table 2. Short-chain fatty acid concentrations (μg/mL) of *S. epidermidis* cultured with various cosmetics.

	Preservative	Soy-Ferment A	Loofah	Natural	Plant	Soy-Ferment B	South Asia	Rice-Ferment A	Rice-Ferment B
0 h									
Succinic	154	190	150	169	180	165	173	173	167
Lactic	169	217	166	182	199	182	367	245	217
Formic	39	49	43	34	35	42	44	52	28
Acetic	238	225	242	242	235	242	236	312	239
Propionic	264	299	293	280	292	284	293	315	289
Isobutyric	110	70	149	130	97	88	136	112	67
24 h									
Succinic	164	200	180	179	201	191	193	166	189
Lactic	1523	1690	1458	1468	1801	1716	1549	1939	2000
Formic	107	93	100	107	80	87	104	88	94
Acetic	357	341	363	362	376	379	379	371	417
Propionic	282	300	301	287	280	277	272	289	278
Isobutyric	167	151	302	232	191	199	335	146	211
48 h									
Succinic	175	199	176	184	191	196	181	207	183
Lactic	1937	2123	1808	1691	2300	2153	1821	2683	2556
Formic	100	95	104	107	103	88	100	119	83
Acetic	408	359	374	397	394	417	391	579	511
Propionic	276	282	257	294	301	265	297	321	283
Isobutyric	184	156	202	213	185	161	184	147	228
0 h with cosmetics									
Succinic	143	179	144	153	169	179	157	169	167
Lactic	147	237	177	193	190	158	183	236	233
Formic	50	52	32	46	38	42	56	44	33
Acetic	218	230	226	231	229	227	233	260	283
Propionic	306	293	279	294	280	276	292	303	294
Isobutyric	109	49	166	103	79	69	117	98	94
24 h with cosmetics									
Succinic	165	174	158	166	188	184	159	181	161
Lactic	1386	1924	1311	1772	1970	1838	1542	2566	2139
Formic	84	79	88	85	93	70	93	84	72
Acetic	331	281	354	309	339	330	372	397	383
Propionic	280	252	257	256	288	274	304	349	278
Isobutyric	200	125	241	280	157	188	255	186	183
48 h with cosmetics									
Succinic	155	177	166	157	182	177	162	190	172
Lactic	1430	2299	1717	2174	2151	2433	1965	2854	2678
Formic	61	73	70	91	87	70	94	94	67
Acetic	290	281	358	332	342	347	426	359	389
Propionic	280	247	266	266	294	246	269	282	261
Isobutyric	165	129	313	263	179	127	179	260	172

Media added with 5% *v/v* cosmetics were inoculated with *S. epidemidis*, and short-chain fatty acids were analyzed using HPLC.



(**A**)

(**B**)

Figure 2. Results of principal component analysis of short-chain fatty acids in cosmetics and cultured *S. epidermidis.* Principal component analysis was performed using concentrations of six short-chain fatty acids obtained from HPLC analysis of cosmetics and cultured *S. epidermidis* cultures. (**A**) Score plot. (**B**) Loading plot.

4. Discussion

In this study, we succeeded in establishing a system for classifying the properties of cosmetics based on their effects on *S. epidermidis*, one of the indigenous skin bacteria. By using this system, cosmetics from various backgrounds can be evaluated, surveyed, and typed based on their properties to the skin microbiota.

Among the nine cosmetics investigated in this study, rice- and soy-fermented cosmetics were plotted in close proximity on the score plot; the fact that these cosmetics were located near succinic acid and formic acid on the loading plot suggests that these cosmetics have similar effects on *S. epidermidis*. The fact that these cosmetics were located near formic acid and acetic acid in the loading plot suggests that they are highly effective in promoting acid production towards *S. epidermidis*. In contrast, South Asian cosmetics, loofar cosmetics, natural cosmetics, and plant-based cosmetics formed clusters at positions near isobutyric acid. These results suggest that these cosmetics may have similar properties and may enhance the production of isobutyric acid. Since South Asian cosmetics contain abundant plant-derived ingredients, it was considered that cosmetics with high plant ingredients would be located in the vicinity. There were two bean-fermented cosmetics, but each was located in a different cluster, suggesting that the same bean-fermented cosmetics may have very different properties.

Cosmetics plotted onto the loading plot space generates many insights into the characteristics of the cosmetics. As described above, lactic acid has beneficial effects on the physical properties and immunity of the skin. Succinic acid has been reported to inhibit the growth of *Propionibacterium acnes*, the causative agent of acne, without causing significant irritation to the skin [16]. On the other hand, acetic acid and formic acid are known to be highly irritating to the skin [13]. Based on these reports, we hypothesized that cosmetics showing a positive correlation with lactic acid and succinic acid in the principal component analysis would be highly effective towards *S. epidermidis*, while cosmetics showing a positive correlation with acetic acid and formic acid would be less effective.

The safety of cosmetics is evaluated using several indices, such as skin reactivity, allergic potential, light toxicity, toxicity, and eye reactivity [17]. However, this study evaluated the property of cosmetics by the effects of cosmetics on skin microbiota. Indeed, some patients demonstrate skin inflammation upon local administration of cosmetics, but the cause was not known. These symptoms might be explained by the disorder of skin microbiota in the future, considering the research.

It is now not known if ingredients in cosmetics such as emollients, lubricants, emulsifiers, fatty acids, alcohols, gelling agents, thickeners, and preservatives might affect the growth or metabolism of skin microbiota. This study will shed light on the effect of these constituents on skin microbiota and will enable the design of more functional cosmetics that function on skin microbiota.

Recently, many cosmetics that appeal to the improvement of skin microbiota are commercially available. However, there are no indices that objectively evaluate the competence of cosmetics on skin microbiota. This confused situation gives rise to the survival of low-quality cosmetics and is unbeneficial for consumers. Therefore, this research will be an effective method to evaluate those cosmetics in terms of their function on skin microbiota, which is beneficial for consumers.

The problem of multidrug-resistant bacteria is one of the strongest threats to human beings now. MRSA is generated from *Staphylococcus aureus*, one of the skin microbiota. It is known that *S. aureus* is sensitive to organic acids, including lactic acid, acetic acid, and propionic acid [18]. Indeed, *S. epidermidis* is known to prevent the colonization of *S. aureus* via delta-lysin, esp, and antimicrobial peptide [19–22], and skin microbiota abundant in *S. epidermidis* or *Corynebacterium acnes* is proposed to be healthy [23]. An imbalance in skin microbiota generates nonpathogenic but unfavorable status of the skin, such as dry skin and sensitivity [24]. Moreover, *S. epidermidis* acts on TLRs and enhances the production of antimicrobial peptides, which also leads to the prevention of opportunistic pathogenic bacteria [25]. *S. epidermidis* also prevents adhesion and biofilm formation by *S. aureus* [26]. It is proposed that the lipoteichoic acid of *S. epidermidis* can prevent skin inflammation mediated via TLR2 and TLR3 [27]. Therefore, the regulation of lactic acid by *S. epidermidis* will be a potent approach to the regulation of *S. aureus*, which leads to the prevention of the rise of MRSA.

So far, in vitro on mono- or co-cultures of skin bacteria to measure the effects of cosmetics via growth measurement using qPCR has been described [28]. Studies using 3D skin models have been applied to elucidate the interaction between skin microbiota and cosmetics [29]. These are considered ex vivo models, and the knowledge obtained in this study can be in the future utilized in these ex vivo models. However, this is the first study to extract information on cosmetics using data obtained from in vitro culture of skin microbiota.

This research has several limitations. For example, it did not adopt a 3D-structured skin model [29]. However, when focusing on the metabolism of skin microbiota, this research will provide some information on the properties of cosmetics. Secondly, this research used only *S. epidermidis*. Skin microbiota contains other bacteria, such as *Propionibacterium acnes*, *Corynebacterium acnes*, and *S. aureus*. Therefore, in the future, these bacteria should also be taken into account. The composition of the media is artificial. Media with which bacteria grow in the actual skin pores are triglycerides and amino acids secreted from the sebaceous

glands. However, since the growth using these components requires an enormous amount of time, media containing glucose was used to facilitate the analysis. Therefore, knowledge obtained in this study should take these into account.

Cosmetic ingredients used that are either functional ingredients, such as preservatives, oils, and emulsifiers, or active ingredients impact the skin microbiota and require caution. Indeed, conventional skincare or hygiene products such as soap, gel, and cream contain preservatives and natural and synthetic chemicals that impact microbiota, even if these effects are not fully investigated in detail until now [30,31]. Glycerol, which was contained in one of the products in this study, apparently does not influence the growth of *S. epidermidis* [32]. Since several glycols, such as dipropylene glycol, reportedly inhibit the growth of *S. aureus* in conjunction with ethanol [33], these glycols might also have inhibited the growth of *S. epidermidis* in this study. Allantoin reportedly decreased the growth of *S. epidermidis* while it enhanced its biofilm formation [34]. C12–C18 alkyl amido prophyl dimethylamine betaine reportedly did not inhibit the growth of *S. epidermidis* [35]. Phenoxyethanol reportedly does not clearly inhibit the growth of *S. epidermidis* [36]. Disodium edetate reportedly inhibits the growth of *S. epidermidis* [37]. Oligosaccharides and amino acids might enhance growth and short-chain fatty acid production. Preservatives contained in cosmetics might hinder the growth of *S. epidermidis*.

Skin pH varies dependent on the parts of the skin. For example, pHs of the skin of the Axilliary vault and antecubital crease tend to be high. In contrast, the pH of the skin of the glabella and alar crease tends to be low [38,39]. Therefore, the administration of cosmetics and a decrease in pH via *S. epidermidis* should be considered in terms of the control of pH.

Recently, fungi that proliferate in skin pores, such as *Malassezia furfur* [40], cause pancreatic oncogenesis and are a serious threat to human health. Since fungi generally cannot grow in an acidic environment, an increase in short-chain fatty acids by *S. epidermidis* might be favorable to prevent the proliferation of these fungi, which is a significant target of future research.

It was not clear from this study which components of these cosmetics stimulated the production of these acids. However, plant extracts and fermented products contain plant and microbial components and microbial metabolites; these components may have stimulated the production of the short-chain fatty acids by *S. epidermidis*.

Atopic dermatitis is one of the major skin diseases in developed countries. It is caused by skin barrier dysfunction such as (1) deficiency in ceramide, cholesterol, and free fatty acids, (2) deficiency in the differentiation of keratinocyte proteins (filaggrin, loricin, and involcrin), (3) sensitivity to immune stimulus, (4) decrease in antimicrobial peptides, and (5) decrease in the diversity of skin microbiota [41]. Indeed, it is reported that *S. epidermidis* is less, and *S. aureus* is abundant in atopic dermatitis patients. Both *S. epidermidis* and *S. aureus* share the characteristics of biofilm formation, but *S. aureus* is more frequent in atopic dermatitis patients [42]. Therefore, the usage of specific cosmetics might cause the dysfunction of skin microbiota and lead to atopic dermatitis.

The cosmetics market is rapidly growing. The global cosmetics market size was estimated to be USD 375.30 billion in 2022. It is expected to grow at a compound annual growth rate (CAGR) of 5.1% from 2021 to 2030 [43]. Therefore, people in the world will more and more use cosmetics. However, since legal regulations of cosmetics vary greatly depending on the countries, some subjective criteria for evaluation of the influence of cosmetics on the skin microbiota are needed. The method proposed in this study will be one such criterion.

It is reported that with increasing age, at the phylum level, *Proteobacteria* increased and *Actinobacteria* decreased, and at the genus level, *Corynebacterium* increased and *Cutibacterium* decreased in Caucasian women [44]. Therefore, it is hypothesized that the alteration of skin microbiota is related to aging, and the manipulation of skin microbiota might lead to anti-aging.

So far, the effects of cosmetics on skin microbiota have been analyzed. For example, the effects of cosmetics on the growth, virulence, cytotoxicity of HaCat keratinocytes, and

biofilm formation of *S. epidermidis* and *Cutibacterium acnes* have been reported [45–47]. However, there has been no study on the short-chain fatty acids of *S. epidermidis*, and this report is the first study to report it. Although it has a limitation in that it does not necessarily reflect the skin condition, it can be used to classify unknown and new cosmetic products and know if it has a characteristic similar to so far sold cosmetics.

The human body is covered with skin. Different parts of the body are inhabited by various kinds of microbes. For example, *S. epidermidis* is rather rich in sabaceous areas of the skin [7]. Therefore, the knowledge obtained from this study can be applied to such skin areas. In contrast, bacteria such as *P. acnes* and *Corynebacterium tuberculostearicum* are rich in dry, moist, and foot areas of the skin [7]. Therefore, in such areas, these bacteria should be considered.

In summary, a cosmetic pattern map using *S. epidermidis* was established in this study. This novel method can be used to pattern cosmetics and infer their effects on skin microbiota.

Author Contributions: Conceptualization, H.K.; validation, H.K., S.I., K.T. and Y.T.; investigation, K.T., S.I., C.M., N.F., N.H., Y.S., Y.H. and H.D.; writing—original draft preparation, H.K. and K.T., writing— review and editing, H.K., K.T. and Y.T.; visualization, H.K. and N.F.; supervision, H.K.; project administration, H.K.; funding acquisition, H.K. All authors have read and agreed to the published version of the manuscript.

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