Influence of Chemical- and Natural-Based Lotions on Bacterial Communities in Human Forearm Skin

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Purpose of this study was to evaluate the influence of a lotion on the bacterial community in the human forearm skin. The chemical- and natural-based lotions were applied on the left and right inner forearm skins, respectively, of 14 participants, who cleansed forearm skin using sterilized cotton swabs. The germs on cotton swabs were analyzed using libraries of PCR amplicons. The genetic diversity of the bacterial communities detected on the natural-based lotion-applied skin (NLS) was significantly higher than that of the bacterial communities on the chemical-based lotion-applied skin (CLS) in all participants, except two. The diversity was estimated based on operational taxonomic unit (OTU), Chao1, Shannon, and Simpson indices. Bacterial communities obtained from the CLS and NLS were phylogenetically separated into 5 and 3 monophyletic groups, respectively, based on lotion types. The taxonomic distribution of the bacterial communities, which were composed of 198 genera in 14 phyla in the CLS and NLS, respectively, was irregularly and biasedly separated into 2 groups based on the lotion types. Among the 14 phyla, Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria were found to be relatively dominant, and 15 of the 198 genera, including Methylobacterium, Propionibacterium, Pseudomonas, Staphylococcus, Streptococcus, and Bacillus were relatively dominant (>0.5%). The taxonomic distribution of dominant bacterial communities from CLS and NLS was irregularly and biasedly separated without relation to the lotion types. In conclusion, the chemical- and natural-based lotions were responsible for changing or influencing the genetic diversity, phylogenetic separation, and taxonomic distribution of skin bacterial communities.

Key Words: Human skin flora, Taxonomic diversity, Phylogenetic differentiation, Natural lotion

INTRODUCTION

The human skin acts as a physical barrier to retain moisture and to prevent infection by pathogens and contamination by harmful chemicals (1). The bacterial flora that reside on the superficial layer of the human epidermis and the upper parts of hair follicles is known to comprise bacterial species belonging to the phyla Corynebacterineae, Propionibacteriaceae, Micrococccineae, Bacteroidetes, Cyanobacteria, Firmicutes, Staphylococcaceae, and Proteobacteria (2). Skin flora are usually not pathogenic, and are either not harmful to the host (commensalism) or offer a benefit to the host (mutualism). The beneficial bacteria ecologically prevent transient pathogenic organisms from colonizing the skin surface by competing for nutrients, secreting chemical antagonists,
or stimulating the skin immune system (3, 4). *Pseudomonas aeruginosa* is an example of a mutualistic bacterium that can turn into a pathogen and cause a disease if its population density on the skin surface increased (5, 6). The organic and inorganic compounds present in eccrine, apocrine, sebum, and dead corneum cells may act as sources of nutrition for microorganisms that inhabit or contaminate the human skin, and can cause the generation of microbial colonies on the skin surface (7). Bacterial colonies generated on the skin surface may cause the development of biofilms. Biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa* have been known to produce a signaling molecule (8, 9). Excessive increase in the biofilms of some bacterial species could cause the expression of a number of virulence genes that convert nonpathogen into pathogen (10, 11). However, routine washing or cleaning of the skin has been reported to impede this bacterial colonization (12).

Generally, the civilized human who removes excretions and dead corneum cells using detergents, alternatively applies chemical- or natural-based cosmetics (creams and lotions) to prevent moisture evaporation and maintain skin condition. Chemical-based cosmetics that contain relatively more chemicals than bio-compounds may impede microbial growth and extend its shelf-life (13). Natural-based cosmetics that contain relatively more bio-compounds than chemicals are known to have specific functions such as moisture maintenance, wrinkle suppression, melanogenesis inhibition, and antioxidation (14). Removal of secretions and corneum cells from human skin by the application of chemical-based lotions could inhibit the development of biofilms and the increase in bacterial density (15). However, the application of natural-based cosmetics could provide better nutritional conditions for the growth of microorganisms (16).

Aging causes the drying of skin surface, change in the skin structure due to decrease in lipid content, and weakening of the skin barrier function, by which the skin becomes more susceptible to infection (17, 18). Age-dependent drying of the corneum also impairs the barrier function by increasing the epidermal proliferation and altering the epidermal structure (19, 20). In order to control for these factors, all the participants in this study were young adults in their early twenties.

The purpose of this study was to evaluate the variations of the skin bacterial communities caused by the application of chemical- and natural-based lotions. Some ingredients of cosmetics (lotions) cause some of skin bacteria to be proliferated or suppressed, by which harmful bacteria or beneficial bacteria for skin health can be increased or decreased. Interests of general peoples for the cosmetics are focused on the effect for beauty care rather than skin health. However, scholarly interest for cosmetics is required to seek the skin health. In order to monitor the bacteria influencing skin health, the skin bacterial communities are required to be metagenomically profiled. For this, the genetic diversity, phylogenetic separation, and taxonomic composition of the bacterial communities detected from the lotion-applied skin surfaces were metagenomically profiled.

### MATERIALS AND METHODS

**Lotion type**

Both chemical-based and natural-based lotions produced by the domestic companies that are The Face Shop and Cell Trion, respectively, were purchased from cosmetic shops. Ingredients present in the chemical-based lotion comprised 27 chemical or chemically semi-synthesized compounds and 5 bio-compounds, while those in the natural-based lotion composed of 25 bio-compounds and 22 chemical or chemically semi-synthesized compounds, as shown in Table 1.

**Experimental participants**

All the participants in this study were 21-24-year old women, who had been using face lotion, body lotion, or hand creams that were chemical- or natural-based for at least 10 years. Fourteen of the participants had used both the lotions before and had not experienced any skin trouble. The participants were free to use the 2 lotions on any part of their bodies, but were instructed to apply the chemical-based lotion on the left inner forearm skin and the natural-based lotion on the right inner forearm skin daily for 15 days during winter season from December to February. This study was a subject to the review exemption by the institutional bioethics committee, which was confirmed by the responsible
Table 1. Ingredients of chemical-based and natural-based lotions sold in cosmetic shops

| Sources                        | Chemical-based lotion                                                                 | Natural-based lotion                                                                 |
|--------------------------------|---------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| Plants, animals, and minerals  | Chamomile flower extract                                                              | Panthenol (Vitamin B5)                                                               |
|                                | Grapefruit extract                                                                    | Plant oil                                                                            |
|                                | Chia seed extract                                                                     | Shea butter                                                                          |
|                                | Hydrogenated lecithin                                                                 | Black currant seed oil                                                               |
|                                | Panthenol                                                                              | Unsaponifiable sunflower seed oil                                                    |
|                                |                                                                                       | Olive oil                                                                            |
|                                |                                                                                       | Balloon vine flower/leaves/vine extract                                              |
|                                |                                                                                       | Sunflower oil                                                                        |
|                                |                                                                                       | Rosemary leaves extract                                                               |
|                                |                                                                                       | Chlorella extract                                                                    |
|                                |                                                                                       | Sea salt                                                                             |
|                                |                                                                                       | Arginine                                                                             |
|                                |                                                                                       | Palmitamide MEA                                                                     |
|                                |                                                                                       | Citric acid                                                                          |
|                                |                                                                                       | Sodium citrate                                                                       |
|                                |                                                                                       | Ceramide NP                                                                          |
|                                |                                                                                       | Hydrogenated lecithin                                                                |
|                                |                                                                                       | Phytoshingsine                                                                       |
|                                |                                                                                       | Tamarind seed polysaccharide                                                         |
|                                |                                                                                       | Phytosterol                                                                          |
|                                |                                                                                       | Glucose                                                                              |
|                                |                                                                                       | Tocopherol                                                                           |
|                                |                                                                                       | Lactic acid                                                                          |
|                                |                                                                                       | Scualane                                                                             |
|                                |                                                                                       | Xanthan gum                                                                          |
| Chemical or chemically         | Dipropylene glycol                                                                     | Glycerine                                                                            |
| semi-synthesized compounds     | Triethylhexanoin                                                                        | Caprylic/capryltri glyceride                                                         |
|                                | Trimethylolpropane tricarpylate/carpnate                                               | Propanediol                                                                          |
|                                | Caprylic/capryltri glyceride                                                           | Ethyl hexyl stearate                                                                 |
|                                | Hydrogenated polydecene                                                                | Cetyl phosphate                                                                       |
|                                | Dimethicone                                                                            | Pentaeerythyl tetraethyl hexanoate                                                    |
|                                | Tri-C14-15 alkylcitrate                                                                | Cetyl alcohol                                                                        |
|                                | Butylene glycol                                                                        | Dimethicone                                                                          |
|                                | Glycerine                                                                              | Decyl ester                                                                          |
|                                | Cetearyl olivate                                                                       | Dicapryl glycol                                                                       |
|                                | Behenyl alcohol                                                                        | Cetyl-1-12-hexanoyl plamitamide                                                      |
|                                | Caprylic/capric glyceride                                                              | 1,2-hexane diol                                                                       |
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Table 1. Continued

| Sources               | Chemical-based lotion | Natural-based lotion |
|-----------------------|-----------------------|----------------------|
| Sorbitan olivate      |                       | Polyglyceryl-10 stearate |
| Penoxy ethanol        | Sodium PCA            |                      |
| Cyclopentasiloxane    | Octyl dodecanol       |                      |
| PEG-100 stearate      | Hydroxy acetophenone  |                      |
| Glyceryl stearate     | Bentonite             |                      |
| Cabomer               | Hydrated silica       |                      |
| Propylene glycol      | Cetearyl alcohol      |                      |
| Hydrogenated phosphatidylecholine| | Carborner |
| Potassium hydroxide   | Disodium EDTA         |                      |
| Sodium alluronate     | Olive oil decyl ester |                      |
| Disodium EDTA         | Flavor                |                      |
| Polyglutamic acid     |                       |                      |
| Cetearyl alcohol      |                       |                      |
| Stearic acid          |                       |                      |
| Flavor                |                       |                      |

professors in the Seokyeong University.

Application of lotions and cleansing

The chemical- and natural-based lotions were applied on left (1L~14L) and right (1R~14R) inner forearm skins, respectively, because the individual and physical conditions of the left and right forearm skin of each participant was not likely to be different. The amount of lotion used by the participants was not controlled; the participants were permitted to spontaneously adjust the amount used based on personal habits. Cotton swabs wetted with saline in conical tubes were autoclaved for 20 min for sterilization. The participants cleansed their left (1L~14L) and right (1R~14R) inner forearms using 10 such sterilized cotton swabs before taking a bath or shower in the evening on the 15th day since they have applied the lotions on their forearm. The cotton swabs used for the cleansing were gathered in the sterilized conical tube immediately after cleansing, while the other end that was used as a hilt was removed as shown in Fig. 1. All the participants previously learned the method for gathering of cotton swabs in conical tube and practically gathered the cotton swabs after cleansing only once on the 15th day.

Metagenomic DNA extraction and pyrosequencing

The ten cotton swabs used for cleansing the inner forearm skins were frozen at -20°C and transported on dry ice to the laboratory at Macrogen (Seoul, Korea), and then used as the samples for metagenomic analysis. DNA was extracted from the bacterial cells using a stool Power water® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, USA). A library was prepared using the PCR products according to the GS FLX plus library prep guide. The emulsion PCR (emPCR), corresponding to clonal amplification of the purified library, was carried out using the GS-FLX plus emPCR Kit (454 Life Sciences, Branford, USA). The 16S universal primers 27F (5’ GAGTTTGA TCMTGGCTCAG 3’) and 518R (5’ WTTACCGCGGCTGCTGG 3’) were used for amplifying the 16S-rRNA genes using the FastStart High Fidelity PCR system (Roche, Basel, Switzerland). After the PCR, the products were purified using AMPure beads (Beckman Coulter, Brea, USA). Sequencing was performed using Roche 454 GS-FLX plus (Basel, Switzerland) at Macrogen.
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Ltd. (Seoul, Korea) using the next generation sequencing technique. All the sequence reads were compared to the Silva rRNA database using BLAST, and the taxonomical hierarchies were assigned based on obtaining more than 97%, 94%, 90%, 85%, 80%, and 75% similarity for species, genus, family, order, class, and phylum, respectively. The CD-HIT-OTU software (Research Group of Weizhong Li, SanDiego, USA) was used for clustering and analyzing the OTU of community richness. The Shannon-Weaver diversity index and the Simpson index of the microbial communities were estimated using the Mothur software (21, 22).

Ecological relationships among bacteria residing skin

Ecological relationships among the bacterial communities obtained from the 28 samples were assessed using beta diversity, based on pairwise Bray-Curtis similarities that reflect the compositional heterogeneity of species (21). A neighbor-joining phylogenetic tree was constructed based on the compositional heterogeneity of the skin bacteria (all bacteria including skin flora residing skin). The species heterogeneity was determined based on the diversity in the variable region (bases 27-518) of the 16S-rRNA gene, which was amplified with the genomic DNA that was separately extracted from the skin bacterial communities (1L–14L and 1R–14R). Phylogenetic relationships were inferred from these alignments using the neighbor-joining method using 1000 replicates for bootstrapping (higher than 50% threshold) in the MEGA4 (Molecular Evolution Genetics Analysis version 4.0) software package (23).

RESULTS

Genetic diversity in skin bacterial communities

A total of 326,331 bacterial 16S rRNA gene sequences were obtained from the 28 samples from the 14 participants after 454 rounds of pyrosequencing. These sequences were filtered to eliminate ambiguous sequences (27,612), short sequences <168 bp (37,261), chimera sequences (7,095), homo polymer sequences (23), and other sequences (97,338). After the filtration, 157,002 valid reads were obtained. The number of valid reads obtained from the bacterial communities sampled from the 14 CLS (1L–14L) varied from 2,201 to 8,718 in a total of 72,586 reads, while those from the 14 NLS (1R–14R) varied from 3,114 to 14,354 in a total of 84,185 reads as shown in Table 2. Phylum and genus numbers of bacterial communities sampled from 14 CLS varied from 3 to 7 and 6 to 24, respectively, while those from 14 NLS varied from 4 to 8 and 16 to 60, respectively. Average phylum and genus numbers of bacterial communities sampled from 14 CLS varied from 3 to 7 and 6 to 24, respectively, while those from 14 NLS varied from 4 to 8 and 16 to 60, respectively. Average phylum and genus numbers of bacterial communities sampled from 14 CLS and 14 NLS were 4.71 and 6.0 and 30.71, respectively (Table 2). Bacterial diversity indices reflecting the OTUs (richness) as well as the Chao1 (richness), Shannon (diversity), and Simpson (diversity) indices of various taxa and lineages in each sample from CLS and NLS are presented in Table 2. The average OTU, Chao1, Shannon, and Simpson indices of samples from CLS was 26.5, 26.7, 3.01, and 0.755, respectively, and those from NLS were 52.14, 53.17, 3.97, and 0.844, respectively. Based on these indices, the richness and diversity of the bacterial communities obtained from NLS were higher than those of the bacterial communities from CLS in all except 2 participants (1L/1R and 11L/11R).

![Figure 1](image-url) A gathering method of cotton swabs for protection or minimization of contamination from the participants' (samplers') hands
Table 2. Diversity indices calculated for skin bacterial communities originated from the chemical-based lotion (L) and natural-based lotion (R)-applied skin of 14 participants

| Sample name | Valid reads | Phylum number | Genus number | OTUs | Chao1 | Shannon | Simpson | Good coverage |
|-------------|-------------|---------------|--------------|------|-------|---------|---------|---------------|
| 1L          | 4,112       | 5             | 23           | 28.0 | 28.0  | 3.8249  | 0.8958  | 1.0           |
| 2L          | 5,049       | 7             | 19           | 22.0 | 22.0  | 2.5625  | 0.6553  | 1.0           |
| 3L          | 8,718       | 6             | 23           | 30.0 | 30.0  | 3.0230  | 0.7228  | 1.0           |
| 4L          | 6,177       | 5             | 26           | 34.0 | 34.0  | 3.3354  | 0.8178  | 0.9998        |
| 5L          | 7,021       | 5             | 21           | 26.0 | 26.0  | 2.9446  | 0.7258  | 1.0           |
| 6L          | 7,947       | 4             | 22           | 28.0 | 28.0  | 3.2458  | 0.8387  | 0.9998        |
| 7L          | 4,772       | 4             | 11           | 14.0 | 14.0  | 2.0702  | 0.5932  | 0.997         |
| 8L          | 3,254       | 4             | 22           | 33.0 | 33.0  | 2.9517  | 0.7128  | 0.9997        |
| 9L          | 2,327       | 2             | 6            | 18.0 | 20.0  | 1.8554  | 0.6030  | 0.9983        |
| 10L         | 6,920       | 4             | 20           | 26.0 | 26.0  | 3.7235  | 0.8944  | 0.9998        |
| 11L         | 5,982       | 5             | 24           | 36.0 | 36.3  | 4.0226  | 0.9149  | 0.9996        |
| 12L         | 2,761       | 7             | 26           | 40.0 | 40.0  | 2.9931  | 0.7503  | 0.9996        |
| 13L         | 2,201       | 5             | 12           | 15.0 | 15.0  | 2.3799  | 0.6662  | 0.9995        |
| 14L         | 6,245       | 3             | 13           | 21.0 | 21.0  | 3.1919  | 0.7841  | 1.0           |

| Sample name | Valid reads | Phylum number | Genus number | OTUs | Chao1 | Shannon | Simpson | Good coverage |
|-------------|-------------|---------------|--------------|------|-------|---------|---------|---------------|
| Total       | 72,586      | 66            | 268          | 371  | 373.3 | 42.1245 | 10.5751 | –             |
| Average     | 5,184.7     | 4.71          | 19.14        | 26.5001 | 26.66 | 3.009  | 0.7554  | 0.9997        |
| Sddev       | 2,020.9     | 1.38          | 6.18         | 7.8421 | 7.7206 | 0.6333 | 0.1064  | 0.0004        |

| Sample name | Valid reads | Phylum number | Genus number | OTUs | Chao1 | Shannon | Simpson | Good coverage |
|-------------|-------------|---------------|--------------|------|-------|---------|---------|---------------|
| 1R          | 6,615       | 4             | 16           | 21.0 | 26.0  | 2.4719  | 0.6928  | 0.9992        |
| 2R          | 5,371       | 5             | 34           | 46.0 | 46.0  | 4.3093  | 0.9055  | 1.0           |
| 3R          | 14,354      | 7             | 60           | 104.0| 104.0 | 5.4602  | 0.9485  | 0.9999        |
| 4R          | 6,245       | 6             | 32           | 51.0 | 51.0  | 3.8148  | 0.8408  | 1.0           |
| 5R          | 4,989       | 6             | 22           | 29.0 | 29.0  | 2.8244  | 0.6686  | 0.9997        |
| 6R          | 4,485       | 8             | 34           | 42.0 | 43.0  | 4.4437  | 0.9210  | 0.9995        |
| 7R          | 4,739       | 6             | 25           | 88.0 | 89.0  | 4.9196  | 0.9298  | 0.9991        |
| 8R          | 7,662       | 4             | 23           | 40.0 | 40.0  | 2.3985  | 0.6526  | 1.0           |
| 9R          | 5,824       | 8             | 43           | 70.0 | 75.0  | 4.9189  | 0.9437  | 0.9991        |
| 10R         | 5,662       | 6             | 38           | 60.0 | 61.5  | 4.5886  | 0.9343  | 0.9994        |
| 11R         | 4,602       | 4             | 17           | 22.0 | 22.0  | 2.3145  | 0.6210  | 1.0           |
| 12R         | 5,009       | 8             | 35           | 49.0 | 49.0  | 4.4627  | 0.9188  | 1.0           |
| 13R         | 3,114       | 7             | 29           | 79.0 | 79.0  | 4.8918  | 0.9400  | 0.9987        |
| 14R         | 5,514       | 5             | 22           | 29.0 | 29.0  | 3.8139  | 0.8971  | 1.0           |

| Sample name | Valid reads | Phylum number | Genus number | OTUs | Chao1 | Shannon | Simpson | Good coverage |
|-------------|-------------|---------------|--------------|------|-------|---------|---------|---------------|
| Total       | 84,185      | 84            | 430          | 730  | 744.4 | 55.6364 | 11.8145 | –             |
| Average     | 6,013,231   | 6.0           | 30.71        | 52.1429 | 53.1714 | 3.9741  | 0.8439  | 0.9996        |
| Sddev       | 2,630.3     | 1.47          | 11.62        | 25.3069 | 25.3536 | 1.0616  | 0.1251  | 0.0004        |

*Stdev: standard deviation
Phylogenetic relationships among skin bacterial communities

Phylogenetic relationships among the bacterial communities (1L to 14L and 1R to 14R) detected from the CLS and NLS of the 14 participants were analyzed based on the 16S-rRNA sequence reads detected from each bacterial community, as shown in Fig. 2. Bacterial communities from the CLS were separated into 5 monophyletic groups (1, 2, 3, 6, and 8) and 3 out-groups (6L, 9L, and 12L), and those from the NLS were separated into 3 monophyletic groups (4, 5, and 7) and 5 out-groups (1R, 2R, 4R, 11R, and 7R). The bacterial communities detected in the CLS and NLS were not distributed into the same monophyletic groups without exception. The bacterial communities other than those in the monophyletic group were distributed into the out-groups without any interrelationship. The out-group 9L was more related to the monophyletic groups 1 and 2, as it consisted of more bacterial communities in these groups than group 3. Similarly, the out-group 11R was more related to the monophyletic groups 4 and 5, as it consisted of more bacterial communities detected from these groups than group 6. The out-groups 12L, 2R, and 7R were more related to the cluster consisting of the monophyletic groups 1 to 6 than to the

Figure 2. Neighbor-joining phylogenetic tree (dendrogram) of bacterial communities originated from the chemical-based lotion (1L to 14L) and natural-based lotion (1R to 14R)-applied skin of 14 participants based on pairwise Bray-Curtis similarity. Branch length in the tree is proportional to the numbers of nucleotide substitutions as measured by the scale bar (5% dissimilarity).
monophyletic groups 7 and 8. Overall, phylogenetic relationship among CLS-origin or NLS-origin bacterial communities was relatively higher than that between CLS-origin and NLS-origin bacterial communities.

**Taxonomic distribution of skin bacterial communities**

The diversity of the bacterial communities obtained from the 28 lotion-applied skin samples (CLS and NLS) were compared at the phylum level based on the taxonomic classifications of 16S-rRNA pyrosequencing reads shown in Fig. 3. A total of 14 phyla, including **Acidobacteria**, **Actinobacteria**, **Armatimonadetes**, **Bacteroidetes**, **Candidatus**, **Chloroflexi**, **Cyanobacteria**, **Firmicutes**, **Fusobacteria**, **Gemmatimonadetes**, **Planctomycetes**, **Proteobacteria**, **Verrucomicrobia**, and others (unclassified), were found to be irregularly and biasedly distributed in the CLS and the NLS samples. **Proteobacteria** was irregularly distributed in all the samples and was found to be the dominant phylum (occupying 39-89%) in 23 of the samples, except for 9L, 12L, 13L, 7R, and 13R. **Firmicutes** was also irregularly distributed in all the samples, and was the dominant phylum (occupying 40-99%) in the samples 9L, 12L, and 7R, and the second most dominant phylum (occupying 10-30%) in 11 samples, including 3L, 5L, 6L, 7L, 8L, 10L, 2R, 3R, 4R,

**Figure 3.** Distribution of the 16S rRNA gene sequences across bacterial phyla in 28 skin bacterial communities originated from chemical-based lotion (1L~14L) and natural-based lotion (1R~14R)-applied skin of 14 participants.
7R, and 9R. Bacteroidetes was more biasedly distributed in the right forearm samples and was the second most dominant phylum (occupying 30–35%) in the samples 12L and 6R. Candidatus was also biasedly distributed in right forearm skin samples. It was found to be the dominant phylum (occupying about 40%) in the sample 13R and the second most dominant phylum (occupying about 30%) in the sample 7R. Actinobacteria was irregularly distributed in all the samples and was the second most dominant phylum (occupying 20–28%) in the samples 4L, 11L, and 10R. Other phyla (unidentified) were selectively distributed only in the right forearm skin samples, and Acidobacteria was detected in 2 left and 3 right forearm samples. In general, the phylum-based distribution patterns of CLS- and NLS-derived bacterial communities were not very different from each other when compared using band patterns in a bar chart. However, the diversity of bacterial communities obtained from CLS and NLS averaged 5.2 and 6.0 phyla, respectively. In addition, the bacterial communities detected from CLS and NLS contained 198 genera, and the diversity observed at the genus level was positively correlated to the taxonomical diversity observed at the phylum level, as shown in Fig. 4. Even though the bacterial communities detected in the 28 samples (CLS and NLS) consisted of 198 genera (Fig. 5), those detected in the individual CLS and NLS samples averaged 18.9 and 29.7 genera, respectively, when counted based on the number of bands in the bar chart. Of the 198 genera, the average occupation rates of 15 genera, Propionibacterium (4.4%), Hydrotalea (1.9%), Tumebacillus (2.9%), Bacillus (3.4%), Staphylococcus (2.7%), Streptococcus (1.6%), Ochro-
bactrum (2.1%), Methylobacterium (26.8%), Sphingomonas (1.8%), Pelomonas (3.8%), Pseudomonas (6.4%), Massilia (1.0%), Cronobacter (1.0%), Escherichia-Shigella (1.0%), and Corynebacterium (0.6%) were higher than 0.5% (Fig. 5).

Figure 5. Taxonomic diversity of bacterial genera (1 to 198) originated from chemical-based lotion (1L ~ 14L) and natural-based lotion (1R ~ 14R)-applied skin of 14 participants. Color bands are legends and genus names are explanation for taxonomic distribution in Figure 4. The average occupation rates of the relatively dominant genera (>0.5%) were quantitatively represented in parentheses.
DISCUSSION

The susceptibility of the human skin for being colonized by bacteria is determined by physical factors such as moisture, temperature, immune status, and excretions, as well as environmental factors such as seasonal variations, light exposure, washing frequency, and cosmetic use (24, 25). The relatively higher temperature and moisture in regions such as the axilla, perineum, and toe webs allows these regions to harbor more microorganisms than the relatively lower temperature and the drier conditions in regions such as the inner forearm, legs, and trunk (26). Nevertheless, the diversity (198 genera from 14 phyla) of bacterial communities detected in the forearm was not significantly lower than that in other regions of the skin; this may be related to the frequency of washes and the thickness of clothing used in the winter seasons (27). A number of factors thus cause the bacterial habitats on human skin to be widely distributed, which influences the diversity and distribution of skin bacterial communities, including those of the opportunistic pathogens (28). Generally, skin bacteria depend on excretions (sebum, eccrine, apocrine) and dead corneum tissues and are periodically removed along with excretions from the skin, which is dependent on the washing frequency of the host (29). However, skin excretions and washing cannot completely eliminate bacteria from skin. Therefore, personal habits of washing and use of cosmetics may be factors that influence the diversity and distribution of skin bacterial communities (24, 30, 31). In particular, natural-based cosmetics that contain bio-compounds (plant extracts, oils, amino acids, carbohydrate derivatives, vitamins, and organic acids) (Table 1) could mix with the excretions to provide improved sources of nutrition for skin bacteria (15, 32).

Therefore, the bio-compounds that were present in the natural-based lotion would have to be a better source of nutrition for bacterial growth than those in the chemical-based lotion (Table 1). Differences of bacterial growth between samples from the CLS and NLS could have been caused by differences in the genetic diversity between these 2 groups. Transient application of a specific lotion to human skin may not cause a change in the skin condition, but long-term and continuous application of nutritionally different lotions may cause such changes (33). However, the physical characteristics of the left and right forearm skins of one person would not be different by the application of chemical- and natural-based lotions because the autonomic nerves and the endocrine system would physiologically regulate the body homeostasis (34). Thus, growth of bacterial communities on the left and right forearm skins may be influenced by both 14 different physical conditions and 2 lotion types because the forearms of the 14 participants were neither controlled nor changed in any way, except for the application of the 2 lotions (35). It may be assumed that the difference in the nutritional condition changed the ecological condition of the forearm skin, and that this change in the ecological condition in turn caused a change in the bacterial diversity and a phylogenetic separation of bacterial communities based on the type of lotion (nutritional condition) used. Generally, richness and diversity indices of bacterial communities sampled from CLS and NLS of 12 participants except 2 participants (1L/1R and 11L/11R) were proportional to the phylum and genus number and generally higher by application of natural-based lotion than chemical-based lotion but those of the 2 participants were reversed (Table 2). This reversed richness and diversity indices are assumed to be caused by the opportunistic or temporarily variation of CLS or NLS condition or both CLS and NLS. However, the taxonomic distribution of the bacterial communities may not be proportional to the genetic diversity and the phylogenetic differentiation, because the physical conditions of the 14 participants would not be physiologically identical (36, 37).

According to previous studies on human skin flora, the bacteria most frequently detected in human skin were 20 genera, including *Staphylococcus*, *Corynebacterium*, *Streptococcus*, *Propionibacterium*, *Pseudomonas*, *Serratia*, *Acinetobacter*, *Janthiobacterium*, *Halomonas*, *Stenotrophomonas*, *Delftia*, *Comamonas*, *Corynebacterium*, *Kocuria*, *Microbacterium*, *Clostridium*, *Sphingobacterium*, *Chryseobacterium*, and *Acidobacteria* in 8 phyla, including *Proteobacteria*, *Bacteroidetes*, *Propionibacteriaceae*, *Corynebacteriaceae*, *Actinobacteria*, *Firmicutes*, *Staphylococcaceae*,
and Cyanobacteria (2, 5, 7, 24, 37). Among these, we detected 198 genera including 12 skin flora, Staphylococcus, Streptococcus, Pseudomonas, Propionibacterium, Corynebacterium, Stenotrophomonas, Corynebacterium, Comamonas, Micrococcus, Serratia, Halomonas, and Clostridium, and 14 phyla including 4 skin flora Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria from the 28 samples studied; this level of diversity was significantly higher than that reported in previous studies. Candidatus was not generally detected from human skin but detected from at least 8 NLS and 1 CLS in this study. This selective distributions of Candidatus in the 8 NLS are assumed to be related to the suitability between nutritional condition of natural-based lotion and physiology of Candidatus. The taxonomic differences between the bacterial communities detected in the human skin in this study and those detected in previous studies may explain the taxonomic diversity between the bacterial communities detected in the left and right inner forearm samples, as well as the clustering of bacterial communities detected from 28 individual samples (Fig. 3 and 4).

Generally, cosmetics are composed solely of chemical compounds or are prepared by mixing chemical and biological compounds. Normally, ingredients containing both chemical and biological compounds would not be hazardous or toxic. In particular, ingredients like amino acids, fatty acids, plant extracts, organic acids, and carbohydrate derivatives present in the natural-based lotions are safe and non-toxic. However, these biological compounds could also serve as nutrients for microorganisms. Differences in the genetic diversity and phylogenetic differentiation of skin bacteria that were analyzed using the 16S rRNA sequence reads showed that the application of chemical- and natural-based lotions caused different changes in the skin bacterial communities. While the changes in the taxonomic diversity caused by the application of chemical- and natural-based lotions would not cause specific problems in the lotion-treated skin, it might disrupt the balance in the commensal and mutual relations between the host and the skin bacteria.

Conclusively, the taxonomic composition and the richness and diversity of skin bacteria were not significantly influenced by the lotion types but might be independently varied considering the physiological character of participants. However, differences of lotions’ ingredients may be one of various factors to influence variation of bacterial communities because lotion is one of the most general cosmetics that is most generally applied by general peoples.

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