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Natural Antimicrobials and Their Effect on the Microbial Population of the Skin Microbiome

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INTRODUCTION

The microbiome of every individual is as unique and distinct as their fingerprint. The skin microbiome is an accumulation of microbial communities that inhabit the skin and are key players in host defense. Commensal microflora on our skin is responsible for maintaining skin health through restoring immunity and communication with the lymphatic system (1). Indiscriminate microbial destruction, used by preservatives, often unintentionally alters the thriving ecosystem of the skin microbiome. The present innovative study investigates variations in the population of microbial species after the application of antimicrobial peptides.

Our research analyzing the activity of the histone deacetylase (HDAC) enzyme has concluded that some naturally derived antimicrobials are able to destroy pathogenic bacteria while maintaining commensal microflora on the skin—supporting the balance of the microbiome and promoting overall skin health (2). HDAC expression was used as an indicator to compare the effects of the skin's microbiome with traditional biocides versus natural antimicrobials. The application of topical antimicrobials altered the levels of HDAC expression and decreased the local population of the microbiome. Although this research suggested HDAC is the channel of communication between microflora and the skin, the messenger of the microbial cross talk has yet to be determined.

In this study, a more conventional approach was used to analyze the effects of the population of species in the skin microbiome. The effect of the microbial population present on the skin with the application of three antimicrobial peptides [*Leuconostoc* radish root ferment filtrate, *Lactobacillus* ferment, and *Lactobacillus* & *Cocos nucifera*

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(coconut) fruit extract] was compared with a negative control (water) and a positive control (triclosan). The microbiome population was determined by DNA extraction, 16S ribosomal RNA (rRNA) polymerase chain reaction (PCR) amplification, and sequencing.

A less conventional approach was used in regard to panel size and evaluation during this study. Large subject panels allow for trend recognition between subjects. However, with the individuality of each person's microbiome in mind, it would be difficult to establish trends within a group of subjects. This study analyzed rRNA gene sequences obtained from one distinct site, the nasolabial folds, of healthy human subjects. The use of one distinct site was to isolate the geographic location of the microbiome, and it allowed for the individual evaluation of microbial change.

BACKGROUND

To understand the importance of this study, it is crucial to recognize some of the common microorganisms located on the skin and the method of bacterial identification. To understand the importance of the protective force of flora due to the constant interaction between commensals and epithelium, it is crucial to investigate some of the common microorganisms located on the skin. These are historically known for their pathogenic role in disease, yet it is proposed that they may offer a means by which they function to positively promote skin health. The beneficial role of microbes on the surface of the skin, unlike digestive probiotics, remains relatively untapped aside from the known roles of these species in protection against other pathogenic and opportunistic invaders.

The species of microorganisms investigated in this study were *Staphylococcus* sp., *Corynebacterium* sp., *Propionibacterium* sp., *Streptococcus* sp., and *Aerobacillus* sp.

One of the most common and abundant microbes located on the skin is *Staphylococcus epidermidis*. This is a Gram-positive bacterium that may have a similar mutual relationship with the skin as most florae in the gut function (3). It is one of the most common isolates of cutaneous microbiota to date (comprises >90% of aerobic flora according to the present research) and typically resides benignly with infection only occurring in pathogenic form when in conjunction with host predisposition or environmental triggers. According to Cogen et al., a significant amount of research has been performed to identify secondary metabolites and has revealed that many strains of *epidermidis* can produce compounds called lantibiotics and other antimicrobial peptides, which can protect the host from unwanted pathogenic invaders.

One of the main roles of commensals is to protect the host, but these significant investigatory studies also provide fundamental insight on the topic of promoting skin health via mechanisms such as Toll-like Receptor (TLR) signaling, keratinocyte response to pathogenic invaders, and their relation to similar microbial species present on the skin. For example, certain strains of *Staphylococcus aureus* on the skin have also been shown to produce other bacteriocins such as staphylococcin, a peptide responsible for growth inhibition of other *S. aureus* strains (3). This has primarily been recognized as a pathogen until now, as new discoveries regarding its protective role have come out to provide more tools to potentially use this flora for alternative health-promoting effects on the skin.

There has been significant research investigating a second bacterium commonly found on the skin, *Corynebacterium jeikeium*. This microbe offers epidermal protection via a mutualistic relationship with the host. It is a ubiquitous and primarily innocuous bacterium that recently has been found to use manganese acquisition to protect from superoxide radicals (3). This is important for cosmetic chemists, as the enzyme superoxide dismutase may also function to prevent oxidative damage in epidermal tissue. Because *C. jeikeium* scavenges iron and manganese, researchers propose it may also serve as a way to prevent colonization by other invaders.

Given the prevalence of skin colonization, the relative rarity of *C. jeikeium* pathogenesis and the unexplored benefits of the bacterium indicate that this microbe probably lives mutually with other microbes and epithelial cells and has more positive than negative effects on the skin (3). By isolating cultures of *Corynebacterium*, it is also assumed that they could be used to prevent or control oxidative damage to the skin. In addition, the *Corynebacterium glutamicum* strain (a *Corynebacterium* with functional capabilities) has the capacity to produce glutamic acid. The production of a compound and the way in which it interacts with the skin could have a potential effect on downstream targets that cosmetic chemists focus on, such as moisturization.

Propionibacterium acnes is often associated with the detrimental effects of acne, as it is well established that both healthy and acne-prone patients are colonized with the bacterium. Acne may be triggered by many intrinsic and extrinsic factors as comprehensive research on the ailment has demonstrated. However, *P. acnes* involvement in inflammation is a relatively minor one. It is proposed that the abnormal growth of this organism, which is often associated with acne blemishes and pustules, might be a side effect of inflammation, rather than the root cause of it (3).

Studies have shown that antibiotics have mostly reduced inflammation in said volunteers affected with acne, whereas only secondarily inhibiting *P. acnes* growth. Because *P. acnes* is present on healthy skin and acne-prone skin alike, the authors suggest that it may serve more as a mutualistic microbe than a pathogenic one (3). These data taken in conjunction with the aforementioned studies suggest that along with the other microbes commonly associated with infection or disease, these may actually have a lower pathogenic potential than initially hypothesized, with minor roles in the true development of the signs and symptoms associated with acne.

Furthermore, researchers have also used mice, immunized with heat-killed *P. acnes*, and subsequently challenged them with lipopolysaccharides. The results showed that these mice had increased TLR4 sensitivity and lymphocyte antigen 96 (MD 2) upregulation, which means that the increased cytokine levels was a direct indication of the detrimental effects of *P. acnes in vivo* (3). This suggests that *P. acnes* has the ability to enable host cells to respond effectively to pathogenic trauma. Cogen et al. proposed that because of this, it is probable that a similar response could be observed if injections of other types of bacteria were used, serving to highlight another potential mechanism. It is theorized that the supply of nutrients found in the ecological environment of *P. acnes*, such as sebum, is a direct exchange for protection against other pathogenic microbes *P. acnes* defends against (3).

Streptococcus and *Pseudomonas*, microbes highly present in the human microbiome, have been studied in terms of their detrimental contributions to infection and disease. However, Cogen et al. now suggest that they may also serve the host in a protective role,

specifically in regard to epithelium interactions. *Streptococcus pyogenes* secretes pore-forming toxins, such as streptolysin O, which are found to promote wound healing *in vitro* via stimulation of keratinocyte migration. Research suggests that sublytic concentrations of this toxin may induce CD44 expression, potentially modulating collagen, hyaluronate, and other extracellular matrix components in the skin (3).

These findings were investigated in mouse models, but support the possibility that *Streptococcus* and some of its metabolites have the potential to be used as a type of probiotic for the skin. Both the tight skin mouse model of scleroderma and other models mimicking fibrosis showed decreased levels of hydroxyproline after treatment with that toxin. Results indicated that activation in the epidermis leads to a potential reepithelialization of wounds in keratinocytes. Streptokinase is also being considered for clinical use in therapeutic fibrinolysis, according to the *British Journal of Dermatology* (3). Although these toxins secreted by this bacterium may be harmful in large doses, in a tissue-specific context, researchers implore that limited expression of *S. pyogenes* factors may help rather than harm the host. The protective role of *Pseudomonas*, or *Pseudomonas aeruginosa* specifically, should also be considered despite research supporting its initially intermediate involvement in disease. There are some commercial medications on the market today that use by-products of these microbes, such as pseudomonic acid A (mupirocin). Mupirocin is a topical antibiotic developed from *Pseudomonas fluorescens* to treat infections caused by other pathogenic microbes (3). The development of this antibiotic again not only supports the protective role of this flora but also allows us to transition into the possibility of using such microbes and their by-products to promote healthy skin. A peptide produced by *P. aeruginosa* was also found to have potent antibacterial activity against pathogenic invaders, in a similar light to the *Lactobacilli* organisms described previously. This along with other investigative studies alluding to the protective role of *P. aeruginosa* suggests that commensals such as *Pseudomonas* maintain homeostasis, rather than causing it. Cogen et al. summarized the importance of this well: the ubiquitous presence of these and other commensals may be necessary to not only promote protection from other invading microbes via competitive inhibition and excretory techniques, but they may also serve efficacious roles by promoting overall skin health.

The protective antimicrobial capabilities of *Lactobacilli* have been thoroughly investigated, as the primary function of these lactic acid–producing florae is to protect the host by limiting the growth of other pathogens. These florae are commonly used in food-grade digestive probiotics and protect the gastrointestinal tract. In cosmetics, short-chain peptides derived from the fermentation of this organism may be used in personal care applications. Antimicrobial peptides are relatively short, protein-like compounds that are typically 30–60 amino acids in length (4). These peptides are a type of aforementioned bacteriocin, typically produced by bacteria as a defense mechanism to outcompete other microorganisms that may reside in the same topographic environment on the body. In addition to *Lactobacilli*, the class of lactic acid bacterium can be expanded to include microorganisms such as *Enterococcus*, *Pediococcus*, and *Leuconostoc*. These microbes serve as a type of protective armor for the skin to help combat disease. Prince et al. investigated the role of *Lactobacilli* in protecting the host from infection and found that *Lactobacilli reuteri* specifically protects keratinocytes by competitive exclusion of the invading pathogen from its binding sites on the cells (5).

NATURAL ANTIMICROBIAL EXTRACTS

The need for alternative options to synthetic preservation has risen because of increased public pressure and stricter global regulations. This has led to the development of bacteriocins or novel antimicrobial extracts derived from fermentation products. Bacteriocins provide broad-spectrum antimicrobial protection through fermentation of lactic acid bacteria in defined growth media. Bacteriocins and other antimicrobial extracts derived from lactic acid bacterium target cell membranes of invading pathogens, as they mimic the phospholipid structure of microbial cell membranes. This protective mechanism is partly due to the hydrophobicity of the bacteriocin that allows the compound in the extract to enter the phospholipid bilayer, leading to displaced cations that may cause stress to the microbe such as an osmotic imbalance. These protective mechanisms work in various ways by interfering with multiple pathogenic cell functions that ultimately lead to cell death and decay (3–5). However, these microbes and their bacteriocins or analogs have many other functions, aside from protection alone. Recent research has demonstrated that peptides secreted by lactic acid bacteria have multifunctional skin benefits, such as moisturization and trans-epidermal water loss reduction (6). This offers a path in which commensal flora and intentionally added compounds derived from such, or probiotic by-products, can be used for their efficacious purposes as well.

Leuconostoc radish root ferment filtrate, *Lactobacillus* ferment, and *Lactobacillus* & *Cocos nucifera* (coconut) fruit extract are three antimicrobial extracts capable of providing a cosmetic benefit of moisturization, have the ability to uphold product integrity, and offer an alternative to traditional preservatives. *Leuconostoc* radish root ferment filtrate and *Lactobacillus* ferment provide broad-spectrum antimicrobial protection against bacteria, yeast, and mold. *Lactobacillus* & *Cocos nucifera* (coconut) fruit extract is an antifungal active designed to prevent the growth of yeast and mold. The minimum inhibitory concentration (MIC) for each antimicrobial peptide is shown in Table I.

METAGENOMICS ANALYSIS

16S rRNA sequencing was the method of bacterial identification used in this study. An overview of bacterial identification by 16S rRNA sequencing is shown in Figure 1.

Table I
MIC for *Leuconostoc* Radish Root Ferment Filtrate, *Lactobacillus* Ferment, and *Lactobacillus* & *Cocos nucifera* (Coconut) Fruit Extract

	Minimum inhibitory concentration (%)		
Organism (ATCC #)	*Leuconostoc* radish root ferment filtrate	*Lactobacillus* ferment	*Lactobacillus* & *Cocos nucifera* (coconut) fruit extract
Escherichia coli #8739	2.0	0.5	—
S. aureus #6538	1.0	0.5	—
P. aeruginosa #9027	2.0	0.5	—
Candida albicans #10231	2.0	0.5	0.5
Aspergillus brasiliensis #16404	2.0	0.5	0.5

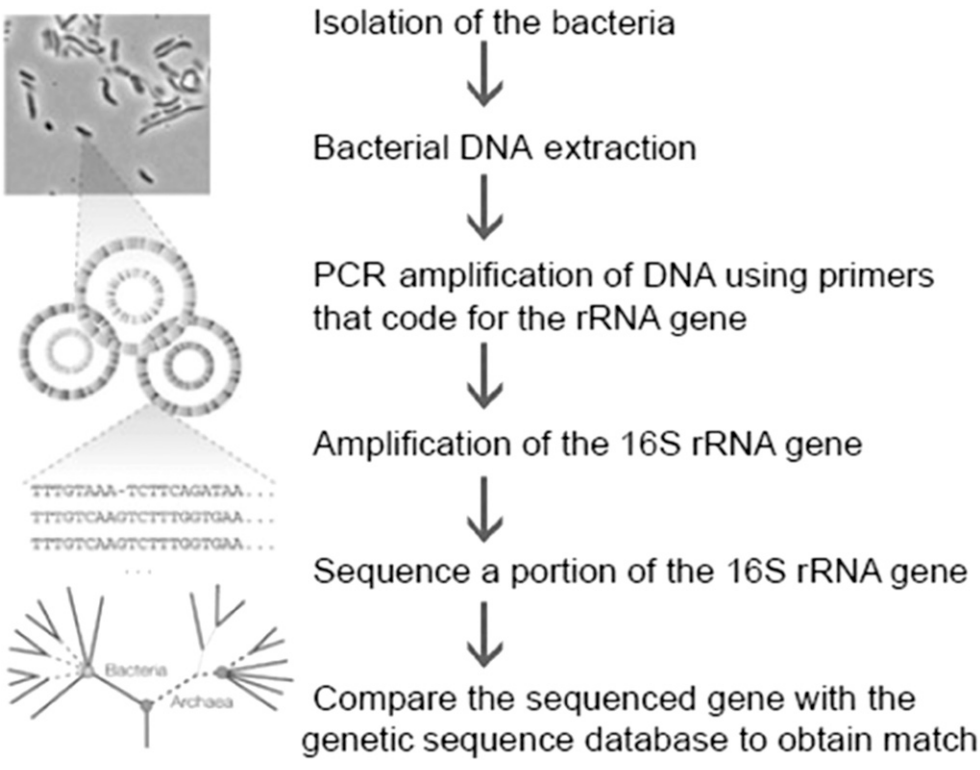

Figure 1. Overview of bacterial identification by 16S rRNA sequencing.

16S rRNA sequencing is a common amplicon sequencing method used to identify and compare bacteria present within complex microbiomes and environments. 16S rRNA sequencing focuses on the analysis of rRNA. Ribosomes are complex structures found in the cells of all living organisms and play a role in protein synthesis. Prokaryotic ribosomes consist of two subunits, a large and a small subunit, with 16S rRNA being a part of the small subunit. The 16S rRNA gene contains hypervariable regions that provide a species-specific signature which is useful for the bacterial identification process (7). rRNA is conserved in cells, and distantly related organisms have remarkably similar portions of 16S rRNA sequences. 16S rRNA gene sequencing

Table II
Changes in Microbiome Population for Participant 1 Treated with *Leuconostoc* Radish Root Ferment Filtrate

Microbiome population	T0	T2	T3
Propionibacterium	5.78E+05	3.41E+04	4.08E+03
Staphylococcus	1.07E+05	1.05E+03	2.32E+02
Aerobacillus	1.05E+04	5.58E+02	4.10E+01
Corynebacterium	2.00E+04	1.01E+03	1.20E+02
Streptococcus	1.89E+04	6.25E+03	1.18E+03

Values expressed in cfu/mL at each timepoint.

Table III
Changes in Microbiome Population for Participant 2 Treated with *Leuconostoc* Radish Root Ferment Filtrate

Microbiome population	T0	T2	T3
Propionibacterium	7.14E+03	8.04E+03	6.57E+03
Staphylococcus	1.05E+03	1.20E+01	2.44E+02
Aerobacillus	5.50E+01	3.00E+00	3.30E+01
Corynebacterium	3.40E+01	3.13E+02	5.10E+01
Streptococcus	1.64E+02	8.10E+01	1.60E+01

Values expressed in cfu/mL at each timepoint.

is commonly used to identify diversities in bacterial microorganisms and study phylogenetic relationships between them (7). There are several advantages of using rRNA in molecular techniques such as gene sequencing. These advantages include the presence of ribosomes and rRNA in all cells, the highly conservative nature of the 16S rRNA gene, and the large size of the 16S rRNA gene, enabling its usage for informatics purposes (8).

The analysis of rRNA genes begins with isolating a sample of bacteria, followed by the extraction of bacterial DNA. The bacterial DNA undergoes PCR amplification using primers that specifically code for the 16S rRNA gene fragment. Amplification produces a population of rRNA gene fragments of equal size, determined by the specific primers used. The population of rRNA gene fragments is considered to be representative of the natural microbial population (8). The amplified rRNA genes are then cloned and sequenced. Comparison of the sequenced rRNA with those in the genetic sequence database allows for the identification of phylogenetic groups (9). The 16S rRNA–based taxonomic characterization can provide information on the microbial population present in a given environment and how their relative distribution may differentially evolve under an applied treatment over time.

MATERIALS AND METHODS

A DNA extraction, 16S rRNA PCR amplification, and sequencing study of the skin was conducted to evaluate the microbiome population present on the facial skin and the respective changes in microbial populations after 2 weeks of product applications.

Table IV
Changes in Microbiome Population for Participant 3 Treated with *Leuconostoc* Radish Root Ferment Filtrate

Microbiome population	T0	T2	T3
Propionibacterium	5.46E+03	2.43E+03	5.98E+03
Staphylococcus	8.02E+02	3.70E+01	2.35E+03
Aerobacillus	8.40E+01	3.20E+01	1.05E+02
Corynebacterium	7.03E+02	1.15E+02	5.44E+02
Streptococcus	1.10E+01	9.00E+00	4.00E+00

Values expressed in cfu/mL at each timepoint.

Table V
Changes in Microbiome Population for Participant 4 Treated with *Lactobacillus* Ferment

Microbiome population	T0	T2	T3
Propionibacterium	3.03E+05	3.40E+03	1.12E+04
Staphylococcus	5.80E+04	5.69E+02	2.98E+03
Aerobacillus	6.76E+03	6.40E+01	1.85E+02
Corynebacterium	6.24E+03	7.63E+02	4.76E+02
Streptococcus	3.06E+02	4.20E+01	4.20E+01

Values expressed in cfu/mL at each timepoint.

Fifteen healthy human subjects were separated into five blind treatment groups, with each of the groups having one of the following products applied twice daily to the lateral nasal folds: 100.0% *Leuconostoc* radish root ferment filtrate, 100.0% *Lactobacillus* ferment, 100.0% *Lactobacillus* & *Cocos nucifera* (coconut) fruit extract, 100.0% water, or 100.0% triclosan. The *Leuconostoc* radish root ferment filtrate, *Lactobacillus* ferment, and *Lactobacillus* & *Cocos* nucifera (coconut) fruit extract are aqueous products and, therefore, are compared with water as the control. It was recommended that the participants not wash their face for 8 h before sampling. Application to the lateral nasal folds was performed by rubbing a premoistened swab back and forth across the treatment area for a total of 60 s. Consistent pressure was applied to the treatment area to ensure substantial recovery of the microbial population. Untreated skin swab samples were taken before product application to serve as a reference for the normal microbial presence on each participant's skin. Each untreated skin swab sample was obtained using a sterile swab premoistened with sterile saline solution.

Treatments were then applied twice a day for a period of 2 weeks, and new samples were taken from each participant to analyze population differences after product applications. One week after the conclusion of product treatments, the last round of samples were taken from each participant to analyze the populations present after treatment ceased. A total of 45 samples were stored in 15 mL conical tubes and frozen at −20°C immediately after sampling. These samples were submitted to the Genomics Laboratory at the David H. Murdock Research Institute (DHMRI) for DNA extraction, 16S rRNA PCR amplification, and sequencing analysis.

The amplicons obtained from PCR amplification from each sample were collected in equimolar proportions into a single pool for sequencing. After sequencing, the samples

Table VI
Changes in Microbiome Population for Participant 6 Treated with *Lactobacillus* Ferment

Microbiome population	T0	T2	T3
Propionibacterium	5.28E+03	5.32E+03	3.11E+03
Staphylococcus	5.41E+02	4.04E+02	1.94E+02
Aerobacillus	6.20E+01	9.00E+01	6.40E+01
Corynebacterium	8.19E+02	5.87E+02	3.76E+02
Streptococcus	1.20E+03	1.54E+03	1.09E+03

Values expressed in cfu/mL at each timepoint.

Table VII
Changes in Microbiome Population for Participant 7 Treated with *Lactobacillus* & *Cocos nucifera* (Coconut) Fruit Extract

Microbiome population	T0	T2	T3
Propionibacterium	1.00E+04	1.02E+04	7.19E+03
Staphylococcus	5.70E+01	9.79E+03	3.39E+03
Aerobacillus	1.60E+01	2.37E+02	1.07E+02
Corynebacterium	0.00E+00	1.95E+03	2.73E+02
Streptococcus	2.65E+02	5.10E+01	7.00E+01

Values expressed in cfu/mL at each timepoint.

underwent taxonomic clustering and analysis. The resultant usable reads were clustered into organizational taxonomic units (OTUs) using the OTU reference database Silva/Arb.

OTUs classify closely distinct microbial organisms from sequences via DNA homology, although, in some cases, they may only read genus or a higher level of taxonomy. It is important to note that OTUs do not always provide specific species for each sequence, but these units still serve as effective indicators of the bacterial diversity on the skin.

Taxonomic clustering and analysis were performed after sequencing the samples to allow for the generation of a phylogenetic tree. The bioinformatics team at DMHRI performed quality assurance (QA) analysis for base-calling quality. In-depth analysis was also performed to analyze the size and nature of 16S rRNA. The data obtained from the readings met the QA specifications for the bioinformatics team to create scoring reads for alignment and database searching. The usable reads were blasted against the OTU reference database (Silva/Arb) to generate OTU abundance results, creating phylogenetic trees and multiple alignments. These results were used to calculate diversity estimates, based on the abundance of microorganism genus in each sample. DMHRI's analysis shows a diverse population with an abundant presence of *Propionibacterium* sp., *Staphylococcus* sp., *Aerobacillus* sp., *Streptococcus* sp., and *Corynebacterium* sp.

RESULTS

The present study determined the microbial population present on the skin and the effect of the microbial population after 2 weeks of varying product treatments. Microbiome

Table VIII
Changes in Microbiome Population for Participant 8 Treated with Lactobacillus & *Cocos nucifera* (Coconut) Fruit Extract

Microbiome population	T0	T2	T3
Propionibacterium	5.93E+03	7.94E+03	5.20E+03
Staphylococcus	5.87E+02	6.90E+02	1.45E+03
Aerobacillus	9.20E+01	7.90E+01	1.00E+02
Corynebacterium	1.14E+02	1.62E+02	2.04E+02
Streptococcus	6.40E+01	1.90E+01	1.68E+02

Values expressed in cfu/mL at each timepoint.

Table IX
Changes in Microbiome Population for Participant 9 Treated with *Lactobacillus* & *Cocos nucifera* (Coconut) Fruit Extract

Microbiome population	T0	T2	T3
Propionibacterium	9.40E+03	8.42E+03	1.74E+03
Staphylococcus	1.48E+02	1.28E+03	4.30E+02
Aerobacillus	1.17E+02	1.05E+02	1.60E+01
Corynebacterium	0.00E+00	8.73E+02	9.50E+01
Streptococcus	5.38E+02	5.38E+02	1.92E+02

Values expressed in cfu/mL at each timepoint.

population was determined after sampling the skin area treated with sterile swabs pre-moistened with sterile sodium chloride solution followed by DNA extraction, 16S rRNA PCR amplification, and sequencing. During the treatment, one of the participants showed high sensitivity to the positive control triclosan; for this reason, the participant did not continue the treatment.

The DNA extracted from the samples that were taken before treatment application began and after the bioinformatic analysis, performed by the bioinformatics team at DMRHI, shows a diverse population as previously mentioned and different populations known as transient and/or opportunistic invaders, such as *Escherichia* sp., *Pseudomonas* sp., *Vibrio* sp., *Clostridium* sp., and *Neisseria* sp. Tables II–XIII show the general microbiome population tendency during the initial, intermediate, and final times of sampling.

Lactobacillus ferment decreased the population of all bacteria genus; *Leuconostoc* radish root ferment filtrate and *Lactobacillus* & *Cocos nucifera* (coconut) fruit extract increased the beneficial bacteria (*Staphylococcus* and *Corynebacterium*) and decreased *Propionibacterium*. After completion of the treatment with the products, *Propionibacterium* sp. reappeared in the skin of the participants using each of the antimicrobial extract treatments. The beneficial bacteria *Staphylococcus* sp., and *Corynebacterium* sp. continued to increase after completion of the treatments.

DISCUSSION AND CONCLUSION

The DNA extraction, 16S rRNA PCR amplification, and sequencing in this present study were conducted to satisfy, in part, the National Institutes of Health Human Microbiome Project: Microbiome Analysis and Sample Collection, and the following

Table X
Changes in Microbiome Population for Participant 10 Treated with Triclosan

Microbiome population	T0	T2	T3
Propionibacterium	2.13E+04	1.49E+04	3.52E+03
Staphylococcus	4.00E+03	1.37E+03	6.97E+02
Aerobacillus	1.78E+02	7.40E+01	3.40E+01
Corynebacterium	0.00E+00	6.52E+02	3.44E+02
Streptococcus	4.30E+01	1.30E+02	2.60E+01

Values expressed in cfu/mL at each timepoint.

Table XI
Changes in Microbiome Population for Participant 12 Treated with Triclosan

Microbiome population	T0	T2	T3
Propionibacterium	1.82E+04	5.34E+03	8.31E+03
Staphylococcus	3.66E+03	8.27E+02	1.86E+03
Aerobacillus	1.07E+02	2.20E+01	8.00E+01
Corynebacterium	4.54E+02	1.22E+02	5.17E+02
Streptococcus	1.07E+03	1.58E+02	8.70E+02

Values expressed in cfu/mL at each timepoint.

DHMRI statements of work: ALCLLC-105 DNA extraction from skin swabs and ALCLLC-106 16S sequencing on DNA extracted from skin swabs.

Under the conditions of this *in vivo* human skin microbiome assay, two of the antimicrobial extracts increased the beneficial bacteria in the participants' skin area studied, while decreasing the presence of *Propionibacterium* sp. *Lactobacillus* ferment decreased all the bacteria genus found in the participants' skin area, compared with triclosan as the positive control and water as the negative control. After completion of the treatment with the products, *Propionibacterium* sp. reappeared in the skin of the participants treated with the antimicrobial extracts. The beneficial bacteria *Staphylococcus* sp. and *Corynebacterium* sp. continued increasing after completion of treatment. By increasing the populations of beneficial bacteria and decreasing the population of *Propionibacterium* sp. (commensal bacteria associated with the development of acne), this present study demonstrates the potential of natural antimicrobials to promote a balanced skin microbiome.

The complexity of the skin microbiome is a relatively new area of research in the cosmetic industry. Since the discovery of microbes on the human body, information on the microbial population has been reported; however, the role of microbial population in skin health is yet to be fully understood. This study adds to the understanding of several dominant microorganisms that, such as *Propionibacterium* and *Staphylococcus* spp., are common components of the skin microbiome and provides insight on the effect of topical application of antimicrobial peptides on the microflora of the skin. Although traditional preservatives may introduce inhibiting factors that directly influence the balance of the skin microbiome, the use of natural antimicrobial peptides may serve as a novel approach to maintaining and promoting healthy skin microbiota. However, further metagenomics analysis is needed to reveal the complex functions and interactions of these antimicrobial extracts and the skin microbiome, as well as the complex functions and interactions

Table XII
Changes in Microbiome Population for Participant 13 Treated with Water

Microbiome population	T0	T2	T3
Propionibacterium	1.93E+04	2.56E+03	1.18E+03
Staphylococcus	4.40E+01	0.00E+00	2.80E+01
Aerobacillus	4.28E+02	2.80E+01	2.10E+01
Corynebacterium	1.07E+03	1.80E+02	3.70E+01
Streptococcus	1.35E+03	2.37E+02	4.90E+01

Values expressed in cfu/mL at each timepoint.

Table XIII
Changes in Microbiome Population for Participant 15 Treated with Water

Microbiome population	T0	T2	T3
Propionibacterium	2.07E+03	5.09E+03	4.12E+02
Staphylococcus	2.00E+00	7.00E+00	0.00E+00
Aerobacillus	6.10E+01	1.28E+02	1.20E+01
Corynebacterium	1.38E+02	3.51E+02	1.80E+01
Streptococcus	1.30E+02	1.50E+01	0.00E+00

Values expressed in cfu/mL at each timepoint.

between the microorganisms of the skin microbiome. Further research in this area could provide more comprehensive approaches to the development of topical products that consider the integral contributions of skin microbiome.

REFERENCES

(1) D. Erturk-Hasdemir and D. Kasper, Resident commensals shaping immunity, *Curr. Opin. Immunol.*, **25**, 450–455 (2013).
(2) M. Danaher, D. Scholz, E. Segura, and M. Darley, Natural vs. synthetic antimicrobials and HDAC as an indicator of microflora health, *Cosmet. Toiletries*, **130**, 22–34 (2015).
(3) A. Cogen, V. Nizet, and R. Gallo, Skin microbiota: a source of disease or defence? *Br. J. Dermatol.*, **158**, 442–455 (2008).
(4) M. R. Yeaman and N. Y. Yount, Mechanisms of antimicrobial peptide action and resistance, *Pharmacol. Rev.*, **55**, 27–55 (2003).
(5) T. Prince, A. J. McBain, C. A. O'Neill, *Lactobacillus reuteri* protects epidermal keratinocytes from *Staphylococcus aureus*-induced cell death by competitive exclusion, *Appl. Environ. Microbiol.*, **78**, 5119–5126 (2012).
(6) N. Izawa and T. Sone, "Cosmetic ingredients fermented by lactic acid bacteria," in *Microbial Production: From Genome Design to Cell Engineering*, H. Anazawa and S. Shimizu. Eds. (Springer, Tokyo, Japan, 2014), pp. 233–242.
(7) J. Ouyang, Z. Pei, L. Lutwick, S. Dalal, L. Yang, N. Cassai, K. Sandhu, B. Hanna, R. Wieczorek, M. Bluth, and M. Pincus, Case report: *Paenibacillus thiaminolyticus*: a new cause of human infection, inducing bacteremia in a patient on hemodialysis, *Ann. Clin. Lab. Sci.*, **38**, 393–400 (2008).
(8) J. M. Janda and L. Abott, 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls, *J. Clin. Microbiol.*, **45**, 2761–2764 (2007).
(9) K. H. Wilson, R. B. Blitchinton, and R.C. Greene, Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction, *J. Clin. Microbiol.*, **28**, 1942–1946 (1990).