Analysis of Bacterial Flora on Sensitive Skin using 16S rDNA Sequencing Technology

Hsiao, Tzu-chih[1*]; Rao, Huan-wan[1]; Luo, Lih-Fen[1]
[1] Juwenlee Cosmetics Technology Center, LUO LIH-FEN Group, Fujian, China
* Hsiao Tzu-Chih, No.21, Longxiang Road, Taiwanese Investment Area, Zhangzhou, Fujian
+86 1380664566, and 2517186904@qq.com of the corresponding author

Abstract

Background: 16S rDNA sequencing technology was used to analyze the characteristics of flora of patients with sensitive skin, providing basic data for cosmetics research and application guidance. **Methods:** Forty female volunteers between the ages of 25 and 50 were recruited and divided into two groups: the sensitive group (A) (n = 18) and the control group (H) (n = 18). Swabs were used to collect skin samples of the volunteers' cheeks, and 16S rDNA sequencing technology was used to detect the skin flora. Computer software was then used to analyze the data. **Results:** A total of 86,525 valid sequences were detected by sequencing technology, and 2,005 OTUs were obtained by sequence clustering, and a total of 1,190 OTUs were annotated to the genus level. The α diversity analysis of skin flora showed that there was no significant difference in the richness and diversity between two groups, and the β-diversity analysis of the flora shows that there are significant differences in the structure of the flora. Differential bacteria analysis at the phylum level showed that *Tenericutes* and *Gemmatimonadetes* increased significantly in the sensitive group. At the genus level, the *Ruminococcus* and *Lactobacillus* significantly increased in the sensitive group, while the *Granulicatella* and *Rothia* significantly decreased. The proportion analysis of bacterial flora showed that the ratio of *Cutibacterium* to *Staphylococcus* in the control group was between 1:1 and 4:1 (R = 1 to 4) , while the proportion of *Cutibacterium* to *Staphylococcus* in the sensitive group was R < 1 or R > 4, indicating that the proportion of *Cutibacterium* to *Staphylococcus* was unbalanced, suggesting that R value might be related to skin sensitivity. **Conclusions:** The facial flora of sensitive patients was disturbed which was manifested as changes in the community structure of the flora. Therefore, an increase in the *Tenericutes* and *Gemmatimonadetes*, and an imbalance in the proportion of *Cutibacterium* and *Staphylococcus* may be related to the development of skin sensitivity.

Keywords: Sensitive skin, Bacterial flora, 16S rDNA sequencing, OTUs

Introduction.

The skin is the largest organ of the human body and has a large number of microorganisms colonized on its surface, including bacteria, fungi, viruses, chlamydia, and certain arthropods (such as mites)[1], of which bacteria account for the largest proportion. Under the internal factors (such as cell metabolism, immune regulation, endocrine, sensitive skin, etc.) and external factors (such as ultraviolet rays, pollutants, hormones, allergens, other toxins and other stimuli), the ecological environment is changing gradually. The ecological environment of the skin includes physiological indicators on the skin's surface, such as oil secretion, natural moisturizing factors, pH value, etc., as well as changes in bacterial flora, resulting in damage to the skin barrier, and the appearance of extreme sensitivity. Studies have shown that the distribution of microflora on the human skin barrier is relatively stable[2].

At present, there have been studies on the detection of microorganisms on the skin of patients with atopic dermatitis and allergic dermatitis, but the detection sites were collected from different parts of the body. The subjects recruited for this study form two groups: sensitive skin and normal skin. The difference in bacterial composition between sensitive skin and normal skin provides a theoretical basis for the development of specific skin care products for people with sensitive skin.

Materials and methods.

1.1 Reagents and Instruments

Microbial sampling swab (Shenzhen Meiruier Technology Co., Ltd), MCT-150-L-C 1.5ml centrifuge tube (Axygen), PCR -0.2 C 0.2ml centrifuge tube (Axygen), QubitTM analytical tube (Invitrogen), VAHTS DNA Clean Beads (Vazyme), 0.9% sodium chloride injection (Sichuan Kelun Pharmaceutical Co., Ltd.), Tween20 (Sigma Alorich), Nuclease-Free Water (Beyotime), Phanta Max Master Mix(2x) (Vazyme), Primer dry powder (Shanghai Sangong Bioengineering Co., Ltd), DNeasy PowerSoil Kit (Qiagen), Universal DNA Library Prep Kit (Vazyme), QubitTM dsDNA HS Assay Kit (Invitrogen), DNA 1K Reagent Kit (Perkin Elmer), MiSeq Reagent Kits v3 (Illumina), IMS-30 Ice maker (Lecon), Biosafety Cabinet-1374 (ThermoFisher), Electrophoresis apparatus-DYY-7C (Beijing Liuyi Instrument Factory), High speed refrigerated centrifuge-fresco 21 (ThermoFisher), Gradient PCR instrument-T960A (Shanghai HealForce Instrument Co.), Ultramicro ultraviolet spectrophotometer-Nanodrop OneC (ThermoFisher), Real time PCR-Quantstudio3 (ThermoFisher), Nucleic acid protein quantitative fluorometer-Qubit4.0 (ThermoFisher), Chemiluminescence imaging system ChemiDoc XRS (Bio-Rad), Bioanalyzer -Labchip GX Touch 24 Nucleic Acid Analyzer (Perkin Elmer), Miseq sequencer (Illumina).

1.2 Research Objective

Thirty-six female volunteers, between the ages of 25 and 50 years old (mean age 37.5±12 years) were recruited and divided into two groups: a sensitive group (A) and a control group (H). The subjects in the sensitive group had sensitive skin, including recurrent allergies, (manifested as contact allergies) and were in a non-inflammatory state when the samples were collected; the subjects in the control group had non-sensitive skin with little or no history of contact allergies. Both groups of volunteers were required to have no skin lesions, no immunodeficiency, and no other skin diseases, including symptoms of atopic dermatitis, eczema, or acne; for the prior two months before the study, both groups were to avoid topical or systemic use of antibacterial drugs, hormones, retinoids or immunosuppressants etc. Volunteers were not allowed to use any skin care products, moisturizers, cosmetics, etc. for 12 hours before sampling. This study complies with the requirements of the revised Declaration of Helsinki (2013), and all volunteers understood the purpose and process of the experiment and signed the informed consent form approved by the Institutional Review Board of the Technical Testing Ccenter of Juwenlee (Fujian) Co.,Ltd. in China. Registration number was CNAS L12773, the protocol number was JWLYF2022-01.

1.3 Experimental Method

1.3.1 Sample Collection

Samples were collected from facial skin, specifically the cheek area. The research was conducted in a room sterilized by ultraviolet light (temperature 22 ℃, humidity 60%). During the sampling process, the volunteers were instructed not to talk, to reduce the impact of droplets on the samples, a change of sterile gloves occurred to avoid cross-contamination between samples. Each cheek was swabbed 10 times with a cotton swab dipped in 0.15M NaCl and 0.1% Tween 20 solution, using light pressure. After collection, the swab head was pushed into a sterile collection tube, and stored at -80 ℃ for later use.

1.3.2 Extraction of Genomic DNA and PCR Amplification

The bacterial genomic DNA on the skin surface was extracted using the DNeasy of soil microbial DNA extraction kit. The concentration and purity of total DNA were detected by ultra-micro UV spectrophotometer, and the quality of extracted DNA was detected by 1% agarose gel electrophoresis. Using the upstream primer FP (-TCGTCGGCAGCGTCAGATGTGTATAAGA GACAGCCTACGGGNGGCWGCAG-) and the downstream primer RP (-TCTCGTGGGCTCG GAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-), the bacterial 16SrDNA V3-V4 variable region was amplified by PCR in a 25 μL amplification system, including 2x Phanta Max Master Mix 12.5 μL, 20 ng DNA template, 1 μL each of 10 μM upstream primer and downstream

primer, and dd H_2O to make up to 25 μL. The amplification program was as follows: pre-denaturation at 95 ℃ for 3 min, denaturation at 95 ℃ for 30 s, annealing at 55 ℃ for 30 s, extension at 72 ℃ for 45 s, a total of 28 cycles, and a final extension at 72 ℃ for 5 min. The PCR products were purified with magnetic beads and a magnetic frame, and the purified DNA fragments were quantitatively detected with a nucleic acid protein, quantitative fluorometer and a fluorescence quantitative PCR instrument.

1.3.3 MiSeq sequencing

We used the Universal DNA Library Prep Kit to build a library of purified DNA fragments and added adapters to the sequencing. Then we purified the adapters using the magnetic beads method, and used the Labchip bioanalyzer to control and monitor the quality of the completed library. Different samples were prepared with MiSeq Reagent Kits v3 in the same proportion. Paired-end sequencing was performed on the Illumina MiSeq platform.

1.3.4 Sequencing data processing

We divided each data sample according to the barcode sequence and PCR amplification primer sequence. We cut the barcode and primer sequence, using FLASH (V1.2.7) to splice the readings of each sample. The sequence obtained by splicing was Raw Tags, which required strict filtering to obtain high-quality Clean Tags. We used Uparse software (Uparse v7.0.1001) to cluster the effective tags of all samples, and cluster the sequences into Operational Taxonomic Units (OTUs) with 97% uniformity. Referring to the tags quality control process of Qiime (V1.9.1), the obtained tags were processed to remove chimera sequences, and the obtained tags sequences were compared to the species annotation database. Finally, we removed the chimera sequence to obtain the final effective Tags. We used Qiime software to calculate Observed-otus, Chao1, Shannon, Simpson, ACE index, and used R software (Version 2.15.3) to draw the dilution curve, the Rank abundance curve, and perform an Alpha diversity index difference analysis between the two groups, using the T-test and Wilcox test. The Unifrac distance was calculated by Qiime software, and the UPGMA sample clustering tree was constructed. R software was used to draw PCA, PCoA and NMDS graphs, and the β-diversity index was analyzed for differences between the two groups. Parametric and non-parametric tests were performed respectively, using the T-test and Wilcox test. LEfSe analysis was performed using LEfSe software, and the default setting of the LDA Score was 4. To conduct Metastats analysis, R software was used to test permutation between two groups for each classification level: Phylum, Class, Order, Family, Genus, Species, getting the *p* value. From there, we used the Benjamini and Hochberg False Discovery Rate method to correct the *p* value and get the *q*-value.

1.3.5 Structural analysis of cheek flora

Alpha diversity was used to analyze the abundance and diversity of microbial communities. The microbial community abundance included the ACE index and the Chao index. A larger index means a larger number of species in the sample. The diversity index included the Shannon index and the Simpson index. When the Shannon index is larger and the Simpson index is smaller, the sample is a community and the diversity of the sample group is higher. β-diversity analysis was used to compare the differences between the two groups of microbial communities. The principal coordinate analysis (PCoA) was used to display the differences in community structure between two groups. Samples with greater community similarities cluster together, while those with greater community differences separate far apart. We used the β-diversity index and analysis of similarities to analyze the box plot of the different groups, directly reflecting the median, dispersion, maximum and minimum values of the sample similarity. At the same time, we used the T-test, Wilcox rank sum test and the β-diversity of species to see if there were significant differences between two groups. For bacterial group difference analysis, the relative content difference of each bacterial group was compared at phylum

and genus level. The R software was used for this analysis. Annotation results of visualization between species was done with KRONA software, statistical analysis of differences in the structure of dominant bacteria between the two groups.

1.3.6 Statistical analysis

Qiime software was used to calculate Observed-otus, Chao1, Shannon, Simpson, and ACE indices, and the test results were expressed as $x \pm s$ for parametric and non-parametric tests, respectively. The T-test and Wilcox rank sum test were used to detect if the difference in α diversity between the groups was significant statistically. Qiime software (Version 1.9.1) was used to calculate the Unifrac distance, construct the UPGMA sample clustering tree, and analyze the differences between two groups in the β diversity index. T-test and Wilcox rank sum test were used to evaluate whether the differences in β diversity between two groups were significant statistically. In the T-test, $p < 0.05$ is considered to be significant statistically.

Results.

1.1 Sequencing Results of 16S rDNA

After paired-end sequencing, the reads were spliced, and an average of 89,221 16S rRNA V3-V4 region sequences were detected for each sample, and an average of 86,525 valid data were obtained after quality control. The sequences were clustered into Operational Taxonomic Units (OTUs) with 97% identity, and a total of 2,005 OTUs were obtained. Among them, the number of OTUs that could be annotated to the database was 1,893 (94.41%), annotated at the world level was 94.41%, the phylum level was 92.12%, the class level was 90.57%, the order level was 87.13%, the family level was 81.95%, the genus level was 59.35%, and the species level was 23.04%. Among the 36 samples in this experiment, *Actinobacteria, Firmicutes, and Proteobacteria* were dominant species at the phylum level; *Actinobacteria, Bacilli, Alphaproteobacteria* were dominant species at the class level; *Propionibacteriales, Bacillales, Rhizobiales* were dominant species at the order level; The dominant species at the Family level were *Propionibacteriaceae, Staphylococcaceae, Xanthobacteraceae*; the dominant species at the genus level were *Cutibacterium, Staphylococcus, Bradyrhizobium*; the dominant species were *Cutibacterium_acnes, Staphylococcus_epidermidis, Bradyrhizobium_elkanii.* See Figure 1.

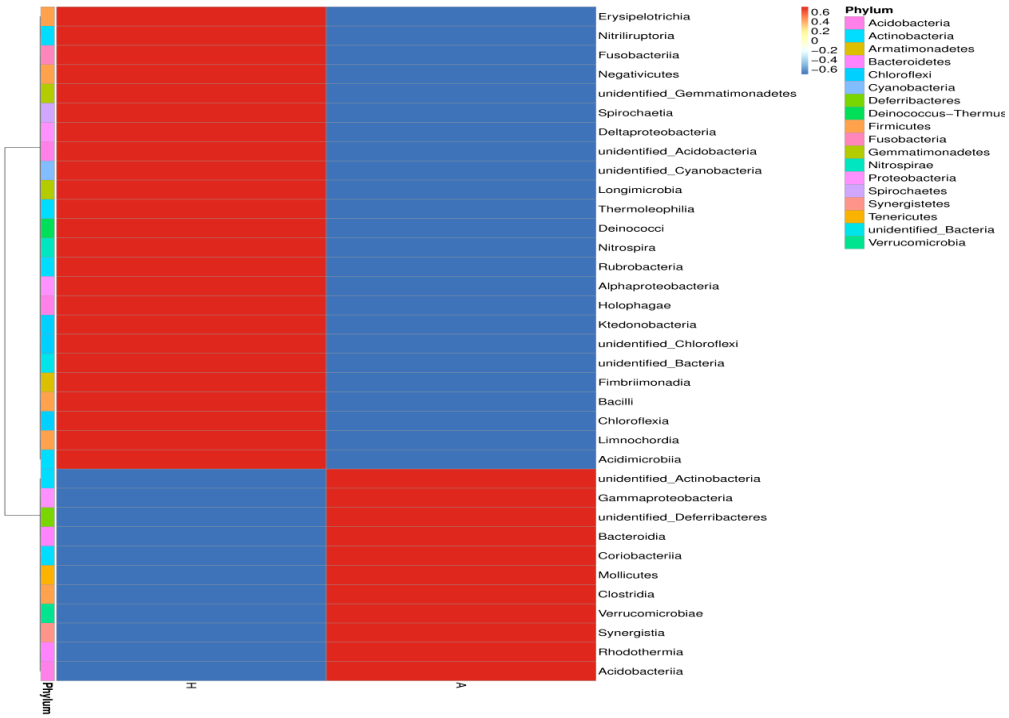


Figure 1 Species abundance clustering diagram

1.2 Bacterial Diversity Analysis

1.2.1 Analysis of Alpha Diversity of Microflora between the Sensitive Group and the Control Group

The Alpha diversity index of different samples under the 97% threshold was analyzed, and the bacterial species richness between the sensitive group and the control group was not statistically significant. Through the Wilcox rank sum test, it was found that the measured number of species had a significant *p* value of 1.0000. The significant *p* value of Shannon's index was 0.8304, and there was no difference in bacterial species diversity between two groups. The degree of α diversity indicates that the abundance and diversity of each bacterial flora in the two groups are similar. Alpha diversity degree of species within the habitat, so therefore, the abundance and diversity of each sample flora in the two groups are similar. See Table 2 and Figure 2.

Group	Abundance index	
	ACE index	Chao index
Control group （H）	485.559±100.933	471.293±99.255
Sensitive group （A）	503.660±136.673	487.186±135.376
p value	＞0.05	＞0.05
Group	Diversity index	
	Shannon index	Simpson index
Control group （H）	2.972±0.518	0.712±0.075
Sensitive group （A）	3.039±0.800	0.686±0.134
p value	＞0.05	＞0.05

Table 2. Comparison of α diversity of facial flora between two groups

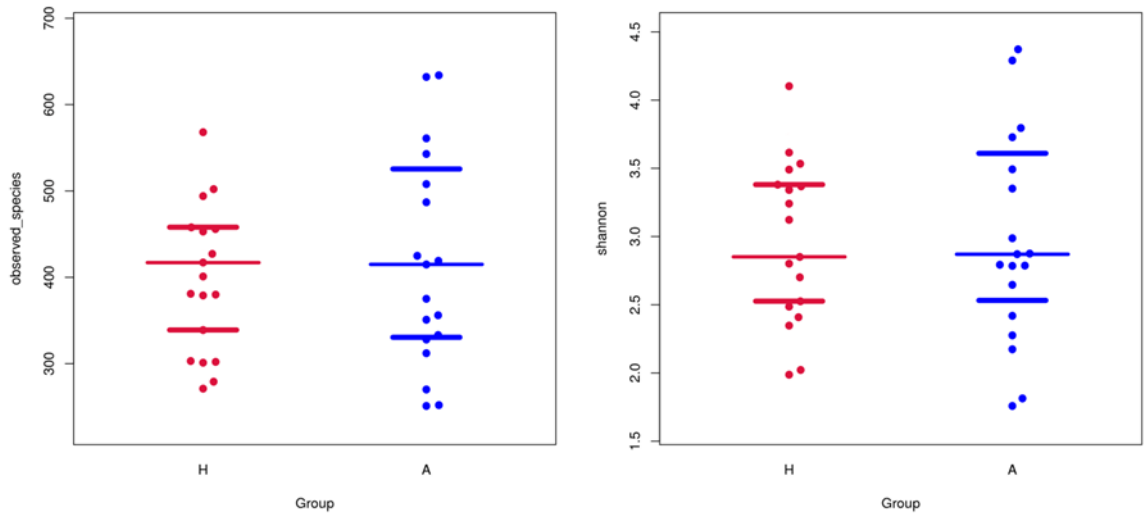
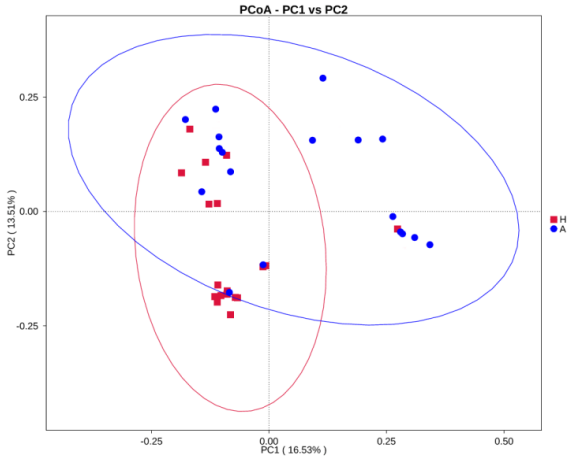


Figure. 2 The bee colony map of the alpha diversity of the facial flora between two groups

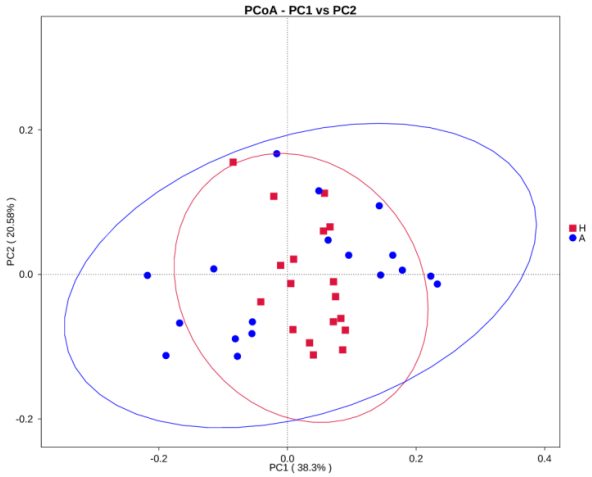
1.2.2 Analysis of β-diversity of Flora between the Sensitive Group and the Control Group

According to the species annotation results of all samples and the abundance information and phylogenetic relationship of OTUs, the Unifrac distance (Unweighted Unifrac) was further calculated. The similarity and variability of the overall microbiome structure of two groups were compared using UniFrac-principal coordinate analysis and UniFrac distance analysis. Both weighted and unweighted UniFrac-PCoA showed that the microbiota structure of the control group was highly similar to each other and tended to aggregate, and the microbiota structure of the sensitive group was quite different. The analysis of T-test and Wilcox rank sum test showed that there were significant

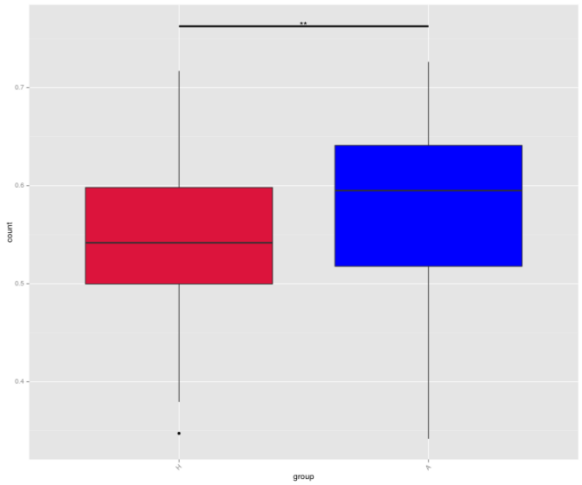
differences in species β diversity between the two groups. Non-Metric Multi-Dimensional Scaling (NMDS) shows that there are differences between samples statistically (Stress = 0.161). See Figure 3.



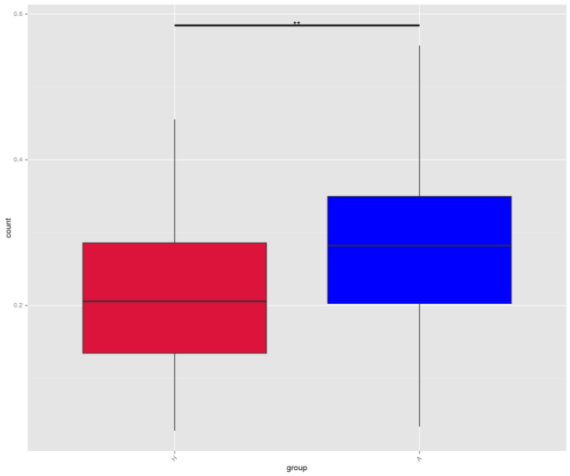
3A Unweighted UniFrac-PCoA



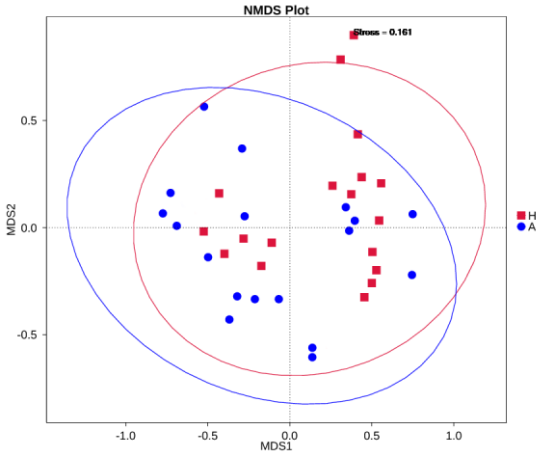
3B Weighted UniFrac-PCoA



3C Unweighted-two_wilcox



3D Weighted-two_wilcox



3E NMDS with cluster

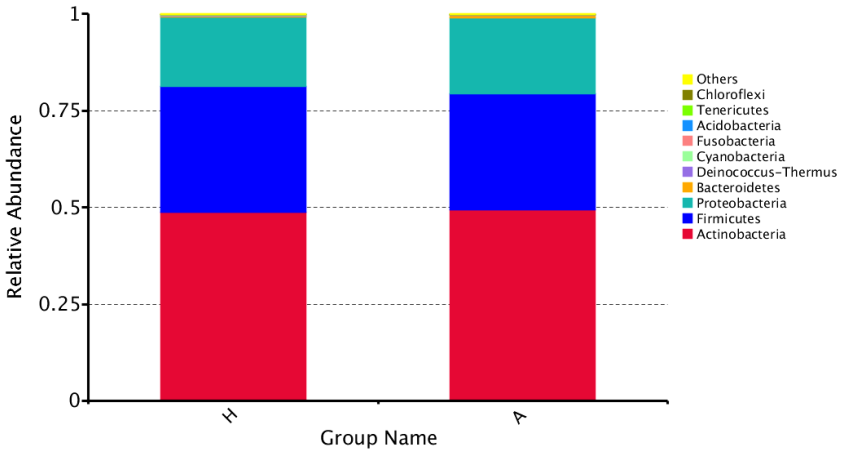
Figure 3 Comparison of the differences in the β diversity of facial flora between two groups

1.3 Differential Bacteria Flora Analysis between the Sensitive Group and the Control Group

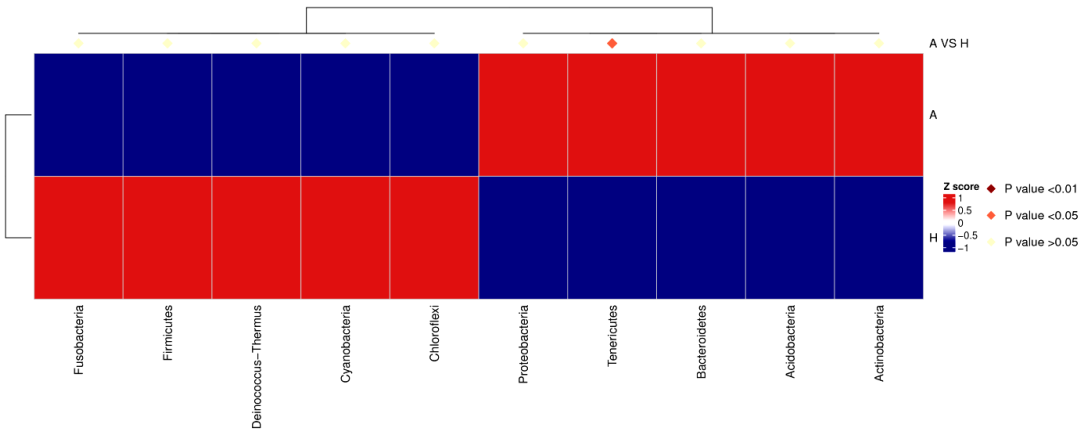
1.3.1 Differential Bacteria Analysis at the Phylum level

The top 10 phyla, by sequencing OTU content, are *Actinobacteria*, *Firmicutes*, *Proteobacteria*,

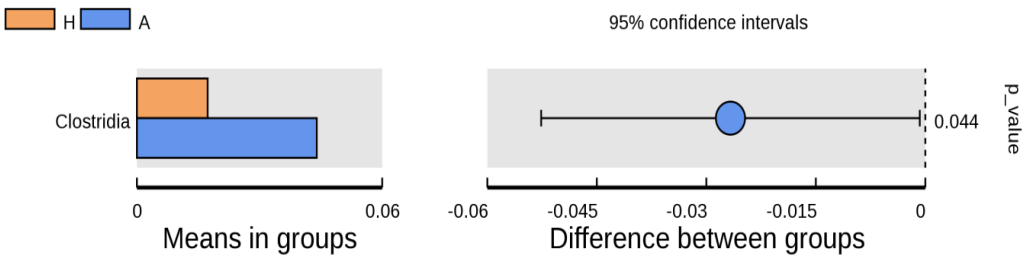
Bacteroidetes, *Deinococcus-Thermus*, *Cyanobacteria*, *Fusobacteria*, *Acidobacteria*, *Tenericutes*, and *Chloroflexi*. *Actinobacteria* were the most dominant in both the sensitive group and the control group, but there was no significant difference in the relative abundance of *Actinobacteria* between the two groups. Hypothesis testing was performed on the species abundance data between two groups using the MetaStat method, and species with significant differences were screened according to the *q* value. The results showed that there were significant differences between two groups in *Tenericutes*, and *Gemmatimonadetes* (*Clostridia*) ($p < 0.05$). See Figure 4.



4A Histogram of relative abundance of species at the phylum level



4B Species annotation heatmap at the phylum level

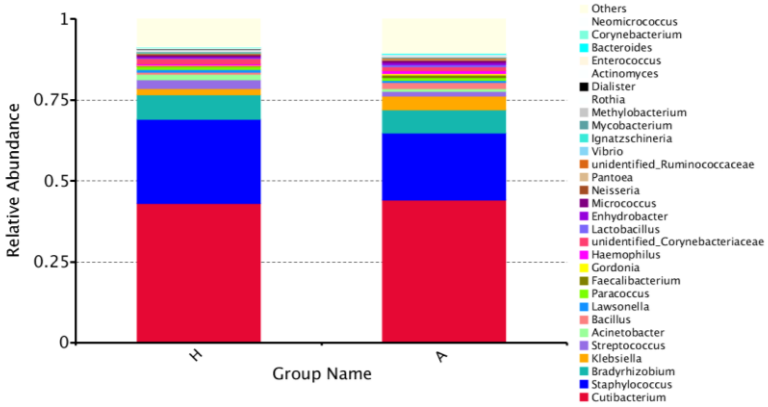


4C T-test analysis of species differences between two groups

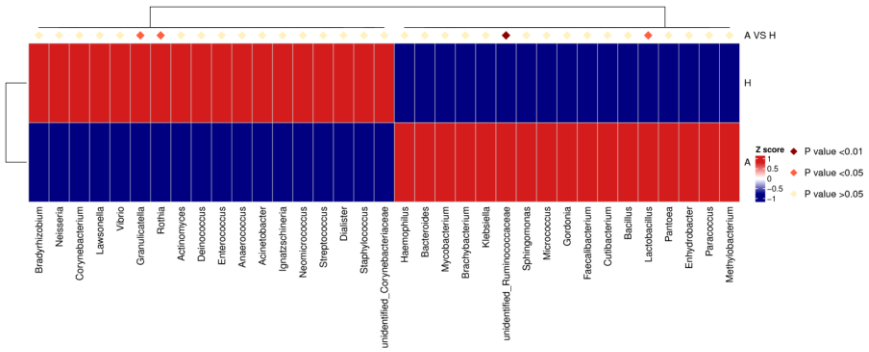
Fig. 4 Analysis of Differential Bacteria between two Groups at the Phylum Level

1.3.2 Differential Bacterial Analysis of Genus-level

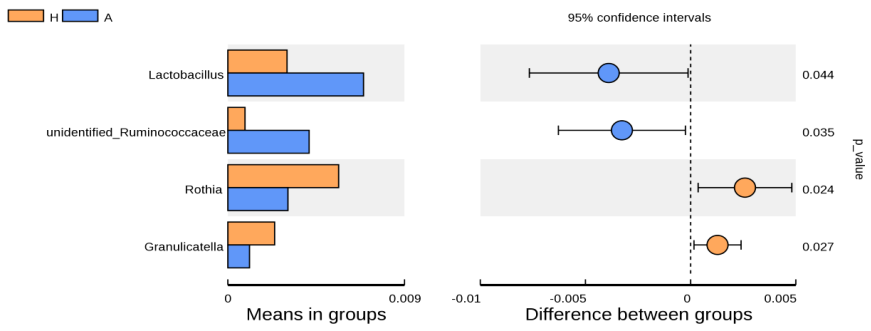
At the genus level, *Cutibacterium*, *Staphylococcus* and *Bradyrhizobium* were the dominant bacteria in both the sensitive group and the control group, but there was no significant difference in the relative abundance of the dominant bacteria between two groups. Hypothesis testing was performed on the species abundance data between two groups using the MetaStat method. Species with significant differences were screened according to the *q* value. The results showed that the control group contained more *Granulicatella* and *Rothia* than the sensitive group, and the difference was statistically significant ($p < 0.05$). The proportion of *Rumenobacter* in the sensitive group was much larger than that in the control group, and the difference between two groups was significant ($p < 0.01$). In addition, the proportion of *Lactobacillus* in the sensitive group was greater than that in the control group, and the difference was statistically significant ($p < 0.05$). See Figure 5.



5A Column chart of relative abundance of species at the genus level



5B Species annotation heatmap at the genus level



5C T-test analysis of species differences between groups

Figure 5. Analysis of differential bacteria between two groups at the genus level

1.4 Analysis of the Relationship Proportion of Bacteria between two Groups

The species annotation results were visualized by KRONA, and the proportion of the annotated genera in each sample of the two groups was analyzed. The results showed that the ratio of the most dominant *Cutibacterium* to the second dominant *Staphylococcus* had a regular distribution between two groups. Specifically, the ratio of *Cutibacterium* to *Staphylococcus* in the control group was R = 1 to 4, while the ratio of *Cutibacterium* to *Staphylococcus* in the sensitive group was R < 1 or R > 4. See Figure 6. It shows that the distribution ratio of *Cutibacterium* and *Staphylococcus* on the skin plays an important role in the balance of skin flora. This leads to a weak microbial barrier on the skin and is vulnerable to external stimuli, resulting in skin sensitivity problems. The R value of one sample in the sensitive group was between 1 and 4. KRONA analysis showed that *Bradyrhizobium* accounted for as high as 42% in this sample (Figure 6). The imbalance of the skin flora overall causes the skin to be sensitive.

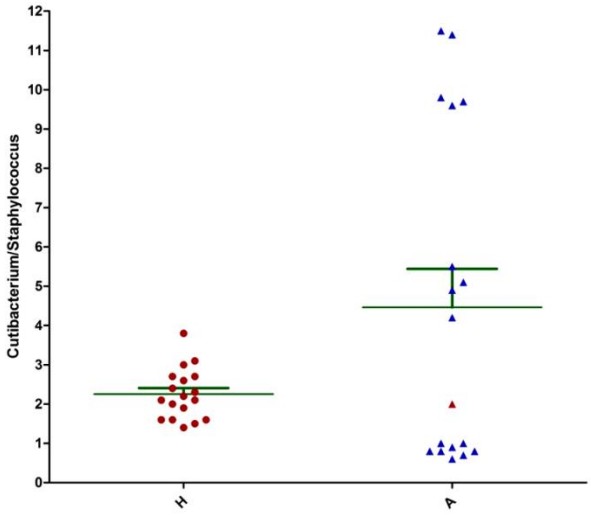


Figure 6 Statistical chart of the ratio of *Cutibacterium* to *Staphylococcus*

Discussion.

The imbalance of microbial homeostasis on the skin's surface, that is, the abnormal pathological relationship between the skin, the environment and the flora, allows the pathogenic bacteria in the temporary flora to absorb into the skin. This compromises the immune regulation of the skin, resulting in a variety of skin diseases, such as atopic dermatitis and eczema, amongst others[3]-[4]. Leyden et al[5] first showed in 1974 that the pathogenesis of allergic dermatitis is related to the structure of the microflora on the surface of the skin. McLoone et al. analyzed the microorganisms by gene sequencing and verified the results of Leyden's test[6]. At present, from the perspective of microorganisms, there are many studies on diseased skin but almost no studies on asymptomatic or simple sensitive skin without disease. The purpose of this study was to analyze the distribution of facial microbes in sensitive skin through High-throughput sequencing technology, and to study the skin flora homeostasis from a microscopic perspective, so as to provide a theoretical basis for the guidance of daily skin care and the research and development of cosmetics.

The study showed that there was no significant difference in the abundance or diversity of facial flora between sensitive skin and normal skin, indicating that the relationship between sensitive skin and flora diversity was not significant. However, patients with allergic dermatitis do present a difference in microbial detection[7]. Since the colonization of microbial flora on the skin has a certain stability, in order to explore the flora status of sensitive skin, there were no skin lesions on the face

of the volunteers in this study, which may be the result of flora diversity. The diversity of flora in sensitive skin differs from those of atopic dermatitis. The β-diversity analysis of facial flora showed that the community structure of sensitive skin was significantly different from that of normal skin, which showed that the sensitive group had a larger proportion of *Tenericutes*, and *Gemmatimonadetes*. In particular, *Clostridium Difficile*, a spore fungus, can cause tissue infections and neurotoxic diseases by secreting exotoxins or invasive enzymes, which can easily increase the sensitivity of the skin to external stimuli.

At the genus level, the proportion of *Ruminococcus* and *Lactobacillus* in the sensitive group was significantly different than that in the control group. From the perspective of intestinal flora regulation, *Lactobacillus* and *Bifidobacterium* are representatives of probiotics[8]-[9], which not only have the ability to resist and inhibit the growth and reproduction of pathogenic bacteria[10], but also improve the host's own immunity and anti-infection resistance to pathogenic bacteria[11]-[12]. However, in the same individual, the composition of the skin microbiota and the gut microbiota were significantly different, with numerous bacteria belonging to the *Actinobacteria* phylum in the skin, but few of the same bacteria in the gut[13]. In addition, the environment surrounding the two bacteria is also quite different. The intestinal tract is an anaerobic environment, while the skin is in an aerobic environment; the skin is often affected by light radiation and is exposed to a complex external environment for a long time. Therefore, whether Lactobacillus has a positive effect on the regulation of skin flora needs further research and analysis.

Statistical analysis of the bacterial groups that accounted for more than 0.7% of each sample found that the ratio of *Cutibacterium* to *Staphylococcus* was within the range of 1:1 and 4:1 (R = 1 to 4) in the control group, while the proportion of *Cutibacterium* to *Staphylococcus* in the sensitive group was distributed outside this range (R < 1 or R > 4). The distribution of the most dominant bacteria (*Cutibacterium*) and the second dominant bacteria (*Staphylococcus*) on the skin affected the microbial barrier function, and the new findings indicate that there is a balance of ratio between *Cutibacterium* and *Staphylococcus*, and the imbalance of this ratio may lead to skin sensitivity. In addition, the R value of one sample in the sensitive group was between 1 and 4 (R = 2). Through the analysis of the bacterial community, it was found that the dominant bacteria in this sample were *Bradyrhizobium* (42%), which may also be a cause of skin sensitivity.

Therefore, the ratio of *Cutibacterium* to *Staphylococcus* is one of the possible causes of skin sensitivity. While, an imbalance between *Cutibacterium* and *Staphylococcus* may lead to skin sensitivity, studies have shown that *Cutibacterium* can produce free fatty acids by hydrolyzing lipids, which can easily cause a skin stress response, indicating that excessive *Cutibacterium* may cause the skin to be sensitive to the external environment. Among the *Staphylococci*, *Staphylococcus aureus* is the pathogenic bacteria, and its delta toxin can cause degranulation of skin mast cells and induce local allergic reactions[14]. *Staphylococcus epidermidis*, of the *Staphylococcu*s group, a symbiotic bacteria on the skin, is usually harmless or even beneficial to the skin. A large number of studies have reported that it plays an important role in maintaining the skin barrier[15]-[16], and can also inhibit an inflammatory immune response caused by *Staphylococcus aureus*[17]-[19]. However, when there is an immune deficiency, it can become a dangerous infection and produce toxic substances-fatty acid modifying enzymes, which break down fatty acids into cholesterol[20]. Therefore, the relative abundance of *Staphylococcus* in the sensitive group was either too high or too low, that is, the increase or decrease of *Staphylococcus aureus* or *Staphylococcus epidermidis* will lead to damage to the skin microecological barrier, resulting in high skin reactivity, poor tolerance and even allergy symptoms.

This study shows that the disorder imbalance of the flora structure may be the cause of skin sensitivity. The pathological mechanism of sensitive skin is that the threshold of external stimuli is lowered[21]. According to the policy of cosmetic skin diseases published by the Ministry of Health, there is a direct relationship between skin sensitivity and excessive skin care[22], which can be caused by complex skin care procedures, including face washing, moisturizing, sun screen, makeup and makeup removal. Excessive cleaning, moisturizing and repairing the skin can also cause skin sensitivity. Therefore, when formulating skin care products for people with sensitive skin, more attention should be paid to the simplicity of active ingredients. One should consider reducing the excessive addition of soap-based ingredients in cleaning products, and reduce the compatibility of polysaccharides, amino acids and other nutrients that are too high in moisturizing or repairing products. Ceramides are highly regarded in moisturizing and repairing products, which are important components of stratum corneum lipids. Their functions include moisturizing, maintaining the stability of stratum corneum structure, and repairing damage[23], but in skin care products for sensitive skin, it is recommended to reduce their proportion in the formula to inhibit the growth of pathogenic bacteria. At the same time, attention must be paid to avoid the addition of glucocorticoids , so as not to cause sensitivity induced by dependent dermatitis. For daily care, facial products can be selected according to the severity and state of skin sensitivity[24], so as to avoid the over-nutrition of the skin environment and further imbalance the flora. There are many studies on the regulation of flora balance focusing on Lactobacillus; the use of Plant polysaccharides such as *Narcissus Tazetta Bulb* Extract, *Dendrobium nobile* stem extract, *Dendrobium officinale* stem extract, Hibiscus taiwanensis S.Y.Hu; Prebiotics such as Alpha-glucan oligosaccharides, Inulin, Fructooligosaccharides (FOS) and Galactooligosaccharides (GOS). The research ideas originate from the regulation of intestinal flora[25], which can also be applied to facial flora. Therefore, attention should be paid to the proportions of ingredients in skin care products, correlating to the distribution of bacteria on the skin.

Conclusion.

This study has certain limitations, as the sample size is relatively small. 16S rDNA sequence analysis technology detected and identified the species of microorganisms that make up the flora on facial skin. In addition, 16S rDNA sequence analysis technology is insufficient for the identification of microbial strains. Therefore, it is necessary to use more advanced detection methods, such as metagenomic sequencing, while also increasing the sample size to further prove the results of this study. To sum up, the maintenance of skin flora homeostasis depends on the application of appropriate skin care products, which can improve skin resistance and reduce skin sensitivity by stabilizing the skin barrier.

Acknowledgments. Thank you to Jennifer de Vetten from Canada- English Language Consultant. The collaboration between the engineers of Luo Lifen Group, Mr. Pan Fa-Wu, Ms. Wang xi-li, Ms. Gao Yuan-Yuan, Ms. Zhang Ya-Fen, Ms. Xie jun-jun for the collaboration.

Conflict of Interest Statement. It is the responsibility of the corresponding author to ensure that any conflict of interest of any of the authors is disclosed. A declaration must be made, if there is no conflict of interest, write NONE.

References.

1. Grice EA, Segre JA (2011) The skin microbiome. Nat Rev Microbiol, 2011, 9(4): 244-253.
2. Julia OH, Allyson LB, et al (2016) Temporal Stability of the Human Skin Microbiome. Cell, 165, 854–866.

3. Belkaid Y, Segre JA (2014) Dialogue between skin microbiota and immunity. Science,346: 954-959.
4. Sanford JA, Gallo RL (2013) Functions of the skin microbiota in health and disease. Semin Immunol, 25(5): 370-377.
5. Leyden JJ, Marples RR, Klgman AM (1974) Staphylococcus aureus in the lesions of atopic dermatitis. British Journal of Dermatology, 90(5): 525-529.
6. Mcloone P, Warnock M, Fyfe L (2015) Honey: a realistic antimicrobial for disorders of the skin. Journal of Microbiology, Immunology and Infection, 54(3): 212-231.
7. Liu X P, Wang XM (2014) Roles of microbiome and skin barrier in dandruff. International Journal of Dermatology and Venereology, 40 (2):106-109.
8. Guo Z, Wang JC, Yan LY, et al (2009) In vitro comparison of probiotic properties of Lactobacillus casei Zhang, a potential new probiotic, with selected probiotic strains. LWT-Food Science and Technology, 42(10): 1640-1646.
9. Zhang Q, Wang JC, Wei AB, et al (2010) Investigation of the growth and survival activities of probiotics Lactobacillus casei Zhang and Bifdobacterium animalis V9 in soymilk. Journal of Dairy Science & Technology, 37(12): 2701-2706.
10. Zhang HP, Ren QJ, Bao QH, et al (2007) The antagonism of Lactobacillus casei Zhang to pathogenic Escherichia coli in mice and the influence on the microbial population in gut Microbiology, 2(1): 2140-2144.
11. Zhang Y, Du RT, Wang LF, et al (2010) The antioxidative effects of probiotic Lactobacillus casei Zhang on the hyperlipidemic rats European Food Research & Technology, 231(1): 151-158.
12. Wu RN, Zhang WY, Sun T S, et al (2011) Proteomic analysis of responses of a new probiotic bacterium Lactobacillus casei Zhang to low acid stress International Journal of Food Microbiology, 147(3): 181-187.
13. Naik S, Bouladoux N, Wilhelm C, et al (2012) Compartmentalized control of skin immunity by resident commensals. Science, 337: 1115-1119.
14. Wollenberg A, Feichtner K (2013) Atopic dermatitis and skin allergies update and outlook. Allergy, 68(12): 1509‑1519.
15. Byrd AL, Belkaid Y, Segre JA (2018) The human skin microbiome. Nature Reviews Microbiology, 16(3): 143-155.
16. Nodake Y, Matsumoto S, Miura R, et al (2015) Pilot study on novel skin care method by augmentation with Staphylococcus epidermidis, an autologous skin microbe-a blinded randomized clinical trial. Journal of Dermatological Science, 79(2): 119-126.
17. Xia XL, Li ZH, Liu KW, et al (2016) Staphylococcal LTA-induced miR-143 inhibits Propionibacterium acnes-mediated inflammatory response in skin. Journal of Investigative Dermatology, 136(3): 621-630.
18. Wang YH, Kuo S, Shu MY, et al (2014) Staphylococcus epidermidis in the human skin microbiome mediates fermentation to inhibit the growth of Propionibacterium acnes: implications of probiotics in acne vulgaris. Applied Microbiology and Biotechnology, 98(1): 411-424.
19. Li DQ, Lei H, Li ZH, et al (2013) A novel lipopeptide from skin commensal activates TLR2/CD36-p38 MAPK signaling to increase antibacterial defense against bacterial infection. PLoS One, 8(3): e58288.
20. Kumar B, Pathak R, Mary PB, et al (2016) New insights into acne pathogenesis: exploring the role of acne-associated microbial populations. Dermatologica Sinica, 34(2): 67-73.
21. Richters R, Falcone D, Uzunbajakava N, et al (2015) What is sensitive skin? a systematic literature review of objective measurements. Skin Pharmacol Physiol, 28(2):75-83.
22. Linder J (2013) Daily care for acne,hyperpigmentation,aging,and sensitive skin. Plast Surg Nurs, 33(4):172-176.

23. Kessner D, Ruettinger A, Kiselev MA, et al (2008) Properties of ceramides and their impact on the stratum corneum structure. Part 2: Stratum couneum lipid model systems. Skin Pharmacol Physiol, 21(2) : 58-74.
24. Nisbet SJ (2018) Skin acceptability of a cosmetic moisturizer formulation in female subjects with sensitive skin. Clin Cosmet Investig Dermatol, 11:213-217.
25. Huang YJ, Marsland BJ, Bunyavanich S, et al (2017) The microbiome in allergic disease: Current understanding and future opportunities-2017 PRACTALL document of the American Academy of Allergy, Asthma & Immunology and the European Academy of Allergy and Clinical Immunology. J Allergy Clin Immunol, 139: 1099-1110.