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

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Poster ID-307

Introduction:

Objective: 16S rDNA sequencing technology was used to analyze the characteristics of sensitive patients' flora with allergic skin, and providing basic data for cosmetics research and application guidance.

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 contact dermatitis(ACD)[3]-[4].

Materials & Methods:

1.1 Skin Samples of the Volunteers

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.

1.2 Extraction，Amplification and High-throughout Sequencing of Microbial DNA

The bacterial genomic DNA was extracted using the DNeasy of soil microbial DNA extraction kit. PCR amplification of bacterial 16SrDNA V3-V4 variable regions using upstream primers FP and downstream primers RP.We used the Universal DNA Library Prep Kit to build a library of purified DNA fragments. Then we used the Labchip bioanalyzer to 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 Sequencing data processing and Structural analysis of cheek flora

We cuted the barcode sequence and PCR amplification primer, using FLASH (V1.2.7) to splice the readings. The sequence obtained by splicing was Raw Tags, which required strict filtering to obtain high-quality Clean Tags. We used Uparse software (V 7.0.1001) to cluster the effective tags, and cluster the sequences into OTUs with 97% uniformity.

1.4 Statistical analysis

Qiime software was used to calculate Observed-otus, ACE、Chao1、Shannon and Simpson indices, and the test results were expressed as x±s for parametric and non-parametric tests. The T-test and Wilcox rank sum test were used to detect if the difference in α diversity was significant statistically. Qiime software was used to calculate the Unifrac distance, construct the UPGMA sample clustering tree, and β-diversity analysis was used to analyze PCoA、box plot、median、dispersion、maximum and minimum values. T-test and Wilcox rank sum test were used to evaluate whether the differences were significant statistically. 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.

Results & Discussion:

1.1 Sequencing Results of 16S rDNA

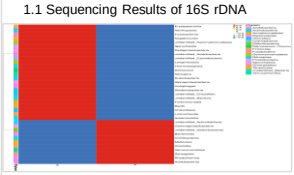


Figure 1 Species abundance clustering diagram

An average of 89,221 16S rRNA V3-V4 were detected, and an average of 86,525 valid data were obtained. The sequences were clustered into OTUs with 97% identity, and a total of 2,005 OTUs were obtained.The number of OTUs annotated at the world level was 94.41%, the phylum level was 92.12%. (Figure 1.)

1.2 Alpha Diversity Analysis of Microflora

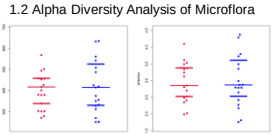


Figure. 2 The bee colony map of the alpha diversity

Group	Richness index	
	ACE index	Chao1 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

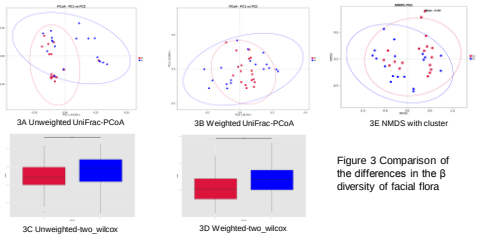
Group	Diversity index	
	Shannon index	Simpson index
Control group (H)	2.972±0.518	0.712±0.075
Sensitive group (A)	3.039±0.800	0.686±0.134
p value	> 0.05	> 0.05

Table 2. Comparison of α diversity of facial flora

The analysis of skin flora α diversity showed that there was no significant difference in the richness and diversity between two groups.

The richness and diversity of each sample flora between-habitat were similar. See Table 2 and Figure 2.

1.3 β-diversity Analysis of Flora between two Groups



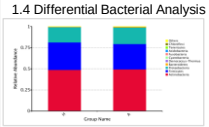
3A Unweighted UniFrac-PCoA 3B Weighted UniFrac-PCoA 3E NMDS with cluster

3C Unweighted-two_wilcox 3D Weighted-two_wilcox

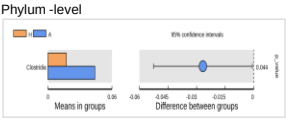
Figure 3 Comparison of the differences in the β diversity of facial flora

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 β diversity analysis of T-test and Wilcox rank sum test showed that there were significant differences in species. Non-Metric Multi-Dimensional Scaling (NMDS) shows that there were differences statistically (Stress = 0.161). See Figure 3.

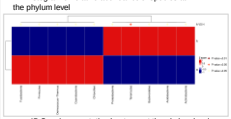
1.4 Differential Bacterial Analysis of Phylum -level



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



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



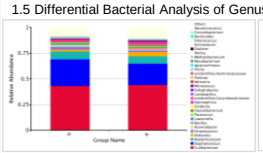
4B Species annotation heatmap at the phylum level

Figure. 4 Analysis of differential bacteria at the phylum level

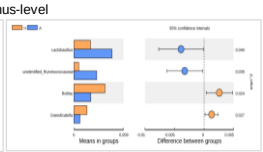
Actinobacteria was the most dominant in the two groups, but there was no significant difference in the relative abundance.

Compared with the control group, the sensitive group had significant differences in *Clostridia* ($p < 0.05$). See Figure 4.

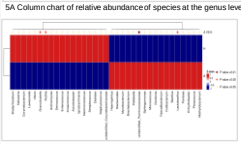
1.5 Differential Bacterial Analysis of Genus-level



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



5C T-test analysis of species differences between two groups



5B Species annotation heatmap at the genus level

Figure 5. Analysis of differential bacteria at the genus level

At the genus level, *Cutibacterium, Staphylococcus and Bradyrhizobium were the dominant bacteria,* the *Lactobacillus* was greater in the sensitive group than the control group, and the difference was significant ($p < 0.05$). The *Ruminococcus was* the significant difference ($p < 0.01$). In addition, *Rothia* and *Granulicatella* was much larger in the control group than he sensitive group was significant difference ($p < 0.05$). Figure 5.

1.6 Analysis of the Relationship Proportion of Bacteria

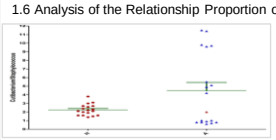


Figure 6 Statistical chart of the ratio of *Cutibacterium* to *Staphylococcus*

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.

Discussion: This study showed that there was no significant difference in the richness or diversity of facial flora between the sensitive group and the control group. The results may be caused by an inclusion criteria error, where subjects with sensitive skin perceive symptoms based on previous experiences, rather than actual symptoms at the time of testing. This differs from acute diseases, such as atopic dermatitis or allergic dermatitis.

Conclusions:

The facial flora of sensitive patients was imbalanced, and showed irregularities in the community structure of the flora. The sensitive group showed a significant increase in *Clostridia* at the phylum level and *Lactobacillus* at the genus level, as well as an imbalance in the proportion of *Cutibacterium* to *Staphylococcus*, all of which may be related to the occurrence and development of skin sensitivity. Therefore, many studies on the balance of flora in sensitive skin focus on prebiotics and the use of plant polysaccharides, such as *Narcissus Tazetta Bulb*, *Dendrobium nobile Lindl.*, *Hibiscus taiwanensis S.Y.Hu*, which could be added to personal care products, but the proportion in product formula should be explored according to the distribution of bacteria, creating products specifically for people with skin sensitivity.

Acknowledgements:

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, Ms. Chen Ying for the collaboration.

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