Facial skin microbiota modulation by *Epilobium fleischeri*, a natural prebiotic plant extract

SC_501

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Introduction:

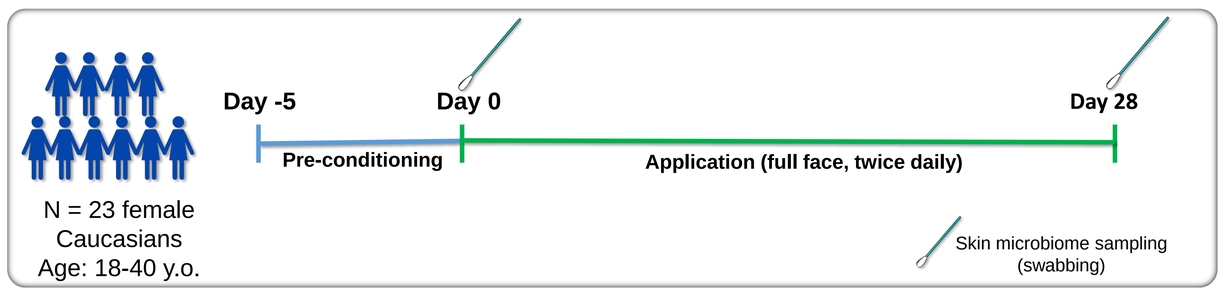
The cutaneous microbiota is being increasingly considered in the cosmetic industry as fundamental to the maintenance of healthy skin. The skin microbiota composition differs highly between body sites [1-3]. Facial skin is a particularly complex environment made of different skin types such as sebaceous (forehead, nose, and chin, also known as T-zone) and dry (cheeks). The composition of the skin microbiota on different facial sites has not been described yet. Therefore, we conducted a clinical study to assess both the bacterial composition on five different facial areas as well as the modulatory effects on the microbiota resulting from the topical application of a plant extract (*Epilobium fleischeri*) known for its sebum regulating properties [4]. In addition, with the outbreak of the COVID-19 pandemic, our investigations on facial skin microbiome are of additional relevance given the emerging need to protect skin from new type of adverse effects falling under the definition of “maskne”, such as skin irritation as well as non-inflammatory (whiteheads and blackheads) and inflammatory acne (papules and pustules), resulting from the widespread use of personal protective equipment (i.e. facial masks) [5, 6].

Materials & Methods:

Test formulations

- Base leave-on formulation (placebo)
- Active leave-on formulation containing 3% of *Epilobium fleischeri* extract.

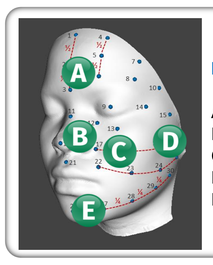
Clinical Study design and microbiome sampling



The clinical study set-up and timeline is shown in the above image. Twenty-three female Caucasians aged between 18-40 with oily skin were enrolled in the study. Informed consent was given by subjects and ethical principles according to Helsinki protocol were followed. A 5 days pre-conditioning phase was carried out before the product testing. During this time, the study participants were provided with a gentle cleanser to be used for cleansing their face. The following application phase lasted 4 weeks during which the products were applied on the face twice daily.

Skin microbiome sampling and 16S rRNA sequencing

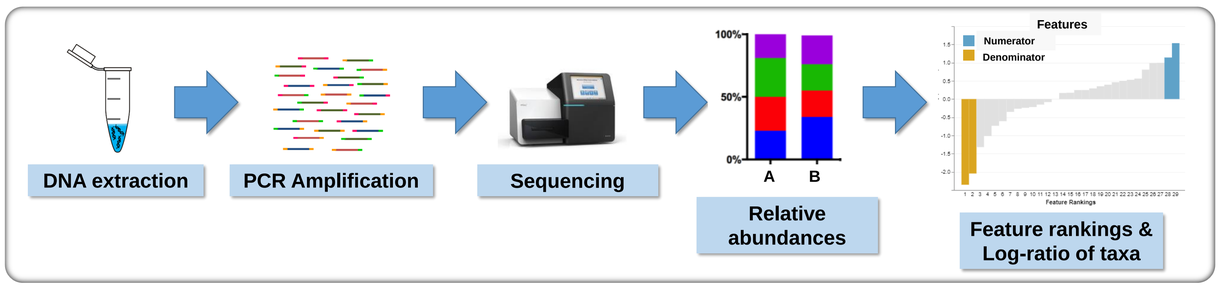
A sterile cotton swab was pre-soaked in a solution containing 0.9% NaCl and 0.1% Tween 20 before being rubbed onto the sampling areas (4cm^2 each).
DNA was extracted from the swab samples and the V3-V4 hypervariable region of the 16S rRNA gene was amplified. The amplicons were sequenced on Illumina’s MiSeq platform with paired end 300 bp reads.



Microbiome sampling areas

A: forehead
B: nose wing
C: front cheek
D: lateral cheek
E: chin

Paired-end sequence reads were collapsed into so-called pseudoreads using sequence overlap with USEARCH version 9.2 [7]. These pseudoreads were collapsed into 97% OTUs. Their classification was performed based on the results of alignment with SNAP version 1.0.23 [8] against the RDP database [9].



Songbird [10] was used to identify feature rankings, and Qurro [11] was used to compute log ratios of these ranked features.

Facial color mapping

Color maps were generated by combining the mean 3D images and the median values of bacteria pairs’ log ratios for each study group. A gradient of blue color was assigned to indicate higher log-ratio values (0 < log-ratio < 2) whereas a gradient of red color was assigned to indicate low and negative log-ratio values (-2 < log-ratio < 0). The changes projected onto a 3D face allow for the identification and visualization of the facial sites in which the microbial shift occurred.

Assessment of porphyrins

Full face images were taken using the imaging system ColorFace® under UV light mode at 365nm to visualize orange fluorescence caused by porphyrins. Fluorescence was quantified via digital image analysis.

Results & Discussion:

Cutibacterium acnes resulted to be the most abundant taxa with a relative abundance ranging from 90% in the forehead, down to 75% in the lateral cheek. The second most abundant bacterium was *Staphylococcus epidermidis* followed by *Corynebacterium kroppenstedtii* which showed its higher relative abundance on the forehead, front and lateral cheek. Less abundant, but in the top 10 microbiota members are *Staphylococcus capitis*, known to colonize facial skin and the scalp, and *Micrococcus yunnanensis* (Figure 1).

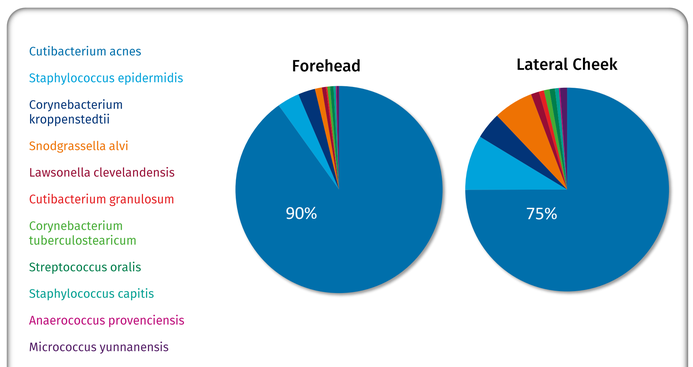


Figure 1. Most abundant OTUs identified on the forehead and on the lateral cheek at baseline, before any product application.

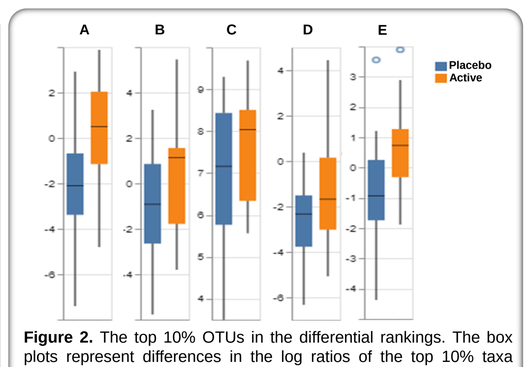


Figure 2. The top 10% OTUs in the differential rankings. The box plots represent differences in the log ratios of the top 10% taxa between the groups after 4 weeks of products application, with the bottom 10% OTUs taken as “reference frames”.

The top 10% features identified in the differential ranking were used to describe the effect of the treatment for all the five facial skin sites considered. An increase in the natural log ratio was observed as compared to placebo in all facial sites in the group applying the active formulation (Figure 2). The data showed a clear shift of the core skin microbiota which was associated to the presence of the *Epilobium fleischeri* extract in the product.

By looking at the differential ranking graphs we could identify key taxa positively associated with the active formulation as well as taxa which showed to be negatively associated. *Staphylococcus capitis* consistently resulted having a low ranking in all the facial sites as compared to the other taxa and was therefore chosen as reference to compute log ratios (Figure 3). These were also made visible via a facial color mapping approach (Figure 4).

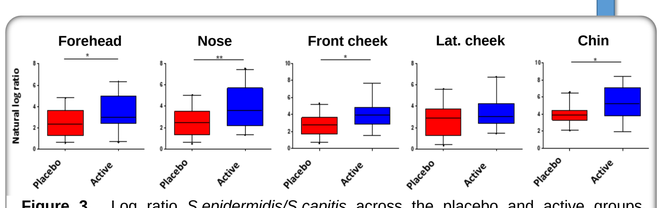


Figure 3. Log ratio *S.epidermidis/S.capitis* across the placebo and active groups. Statistical significance has been calculated via Welch’s t-test (*, p < 0.05; **, p < 0.01).

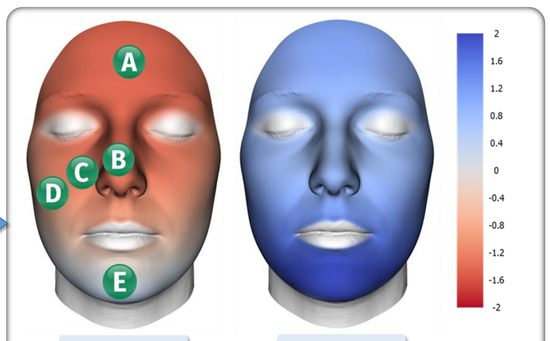


Figure 4. Visualization of log-ratio between *S.*epidermidis/*S.capitis* via facial color mapping. The color maps show log-ratio increase in *S.epidermidis*/ *S.capitis* after 4 weeks treatment with the placebo (left) and with the product (right). Color code (-2 to 2) is shown on the scale on the right-hand column. (A, B, C, D, E were the facial sampling areas).

We also assessed orange fluorescence emission on facial images acquired at baseline and after 4 weeks of treatment for both groups to get a measure of the amount of porphyrins (Figure 5). It could be easily observed an overall significant reduction in porphyrins after 28 days in the cohort applying the *Epilobium fleischeri* extract. In contrast, a strong tendency of increased porphyrin levels could be observed in the cohort applying the placebo.

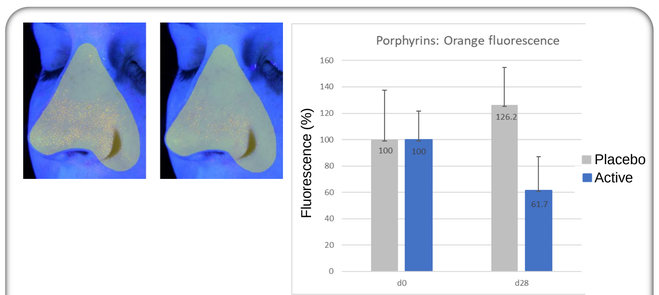


Figure 5. Porphyrins assessment. Left, detail of volunteer n.6 (27 y.o), left profile, with ROI and segmented porphyrins at D0 and D28. Right, fluorescence quantification indicating a significant reduction in the active group after 28 days.

Conclusions:

In summary, our study showed that different facial sites are colonized by different proportions of bacteria, with *C.acnes* being the most abundant, but present in different proportions depending on the biophysical features of the facial skin location i.e., sebaceous area vs dry areas (e.g., forehead vs lateral cheek). Four weeks-long topical application of a natural *Epilobium fleischeri* extract rich in oenothein B resulted in a significant modulation over a series of beneficial facial skin commensals, such as *S.epidermidis*, *S.hominis* and *M.yunnanensis*, providing a beneficial enrichment of these microorganisms in the final microbial composition, while depleting it from opportunistic bacteria such as *S. capitis*, *C. kroppenstedtii* and *C. tuberculostearicum*. Interestingly, we could show a significant decrease in porphyrins on the skin of volunteers in response to the active product. Such evidence would suggest that the natural extract supported a healthier skin phenotype by reducing the secretion of porphyrins by potentially acne-associated *C.acnes* strains (type I strains), even though the overall abundance of *C.acnes* species has not been particularly modulated as compared to other taxa.

Aknowledgments:

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