


ORIGINAL ARTICLE

5- α reductase inhibition by *Epilobium fleischeri* extract modulates facial microbiota structure

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Abstract

Background: Facial skin is a particularly complex environment made of different skin types such as sebaceous (forehead) and dry (cheeks). The skin microbiota composition on different facial sites has not yet been addressed.

Methods: We conducted a 4-week-long, single-centre, randomized and placebo-controlled clinical study involving 23 Caucasian females. We assessed both bacterial composition on five different facial areas and the microbiome modulatory effects resulting from the topical application of a plant extract (*Epilobium fleischeri*). Skin microbiome samples were collected before and after 4 weeks of product application. Microbiota profiling was performed via 16S rRNA gene sequencing, and relative abundance data were used to calculate differentials via a multinomial regression model.

Results: Via ‘reference frames’, we observed shifts in microbial composition after 4 weeks of twice-daily product application and identify certain microbiota species, which were positively associated with the application of the product containing the *Epilobium fleischeri* extract. *Staphylococcus hominis*, *Staphylococcus epidermidis*, and *Micrococcus yunnanensis* appeared to be significantly enriched in the final microbiota composition of the active treatment group.

Conclusion: Facial skin was found to be colonized by a heterogeneous microbiota, and the *Epilobium fleischeri* extract had a modulatory effect on commensal bacteria on the different facial sites.

KEYWORDS

claim substantiation, *Epilobium fleischeri*, microbiota, reference frames, skin care, skin microbiome

Résumé

Contexte: la peau du visage est un environnement particulièrement complexe où l’on trouve des peaux de plusieurs types, par exemple grasse (sur le front) et sèche (sur les joues). La composition du microbiote cutané sur différentes zones du visage n’a pas encore été abordée.

D.I., R.S. and J.C. are all employees of DSM Nutritional Products.

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Méthodes: nous avons mené une étude clinique de 4 semaines monocentrique, randomisée et contrôlée par placebo sur 23 femmes de type caucasien. Nous avons évalué à la fois la composition bactérienne sur cinq zones différentes du visage et les effets modulateurs du microbiome résultant de l'application topique d'un extrait de plante (*Epilobium fleischeri*). Des échantillons de microbiome cutané ont été prélevés avant et après 4 semaines d'application du produit. Un profilage du microbiote a été mené par séquençage du gène de l'ARNr 16S, des données d'abondance relative ont été utilisées pour calculer les différentiels via un modèle de régression multinomiale.

Résultats: nos cadres de référence nous ont permis d'observer des changements de composition microbienne après 4 semaines d'application deux fois par jour du produit et nous avons identifié certaines espèces de microbiote qui ont été positivement associées à l'application du produit contenant l'extrait d'*Epilobium fleischeri*. Les taux de *Staphylococcus hominis*, *Staphylococcus epidermidis* et *Micrococcus yunnanensis* semblaient significativement plus élevés dans la composition finale du microbiote du groupe de traitement actif.

Conclusion: la peau du visage s'est avérée colonisée par un microbiote hétérogène, et l'extrait d'*Epilobium fleischeri* a eu un effet modulateur sur les bactéries commensales des différentes zones du visage.

INTRODUCTION

In context with the recent rise of interest in understanding the role of skin microbiome on skin health, we intended to investigate the microbiota composition of different facial skin sites. The skin microbiome composition is known to vary considerably across body sites and even between individuals [1–3]. Facial skin is heterogeneous and includes areas with high sebum content (the T-zone: forehead, nose and chin) and dry areas where sebum secretion is lower (cheeks). Therefore, it appears evident that facial skin provides different niches for microbial colonization. Some studies on facial microbiome have already been performed; however, to the best of our knowledge, none looked at the microbial composition of different facial sites [4, 5]. Here, we sought to investigate that and to link

microbiota compositional information to the variable sebaceous environments in the face as an example of host-microbe interaction. Moreover, we sought to find out more on the microbial shifts induced after facial application of a cosmetic formulation containing *Epilobium fleischeri* extract, a skin care active ingredient known for its sebum-regulating properties.

Epilobium fleischeri is a rare Alpine species of the Onagraceae family likely to grow in moraines and alluviums close to glaciers. Traditional uses of extracts from various *Epilobium* species are known among others also to treat skin infection. The extracts are known for their anti-proliferative and anti-inflammatory properties mostly due to their rich content in various flavonoids, such as quercitrin, isoquercitrin, myricitrin and isomyricitrin, and in particular the ellagitannin and oenothien B [6]. Moreover,

TABLE 1 Formulations used in the clinical study

INCI Name	Base formulation (Placebo)	Active formulation
	%	%
ACRYLATES/C10-30 ALKYL ACRYLATE CROSSPOLYMER	0.30	0.30
AQUA	Add to 100	
PHENOXYETHANOL, ETHYLHEXYLGLYCERIN	1.00	1.00
GLYCERIN, AQUA, CITRIC ACID, POTASSIUM SORBATE, EPILOBIUM FLEISCHERI FLOWER / LEAF / STEM EXTRACT	0.00	3.00
AQUA, SODIUM HYDROXIDE	Add up to pH 5.5	

it has been shown that oenothein B is a potent inhibitor of 5 α -reductase, an enzyme representing a key target in sebaceous glands known to be involved in the regulation of sebum production. *Epilobium fleischeri* extract has been identified for the high content of oenothein B, and for this reason, the extract finds application in skin care products with sebum-regulating properties [7].

In addition, with the outbreak of the COVID-19 pandemic, our investigations on facial skin microbiome were further supported by the more emerging need to protect skin from new type of adverse effects, 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). The occlusive microenvironment (change in pH, temperature and humidity) generated by mask-wearing and textile-skin friction is thought to lead to microbiome dysbiosis, a microbial imbalance, which is more and more associated with various dermatological conditions [8–10]. Recently, the term ‘maskne’ was coined in order to collectively refer to mask-wearing-associated skin disorders [11]. The rising demand for keeping healthy skin conditions certainly includes that investigations on the composition of the skin microbiota and on its functionality are conducted. Our study, therefore, provides interesting insights into the facial skin microbiota composition on different facial sites, aiming at increasing our overall knowledge on the topic and assessing the microbial modulating effects of topical application of a formulation containing the sebum-regulating *Epilobium fleischeri* extract.

Objectives

The objectives of this clinical study are (1) to investigate the composition of the facial skin microbiota at five different facial sites and (2) to assess the microbial modulating effects resulting from the topical application of a formulation containing the sebum-regulating *Epilobium fleischeri* extract.

MATERIALS AND METHODS

Test formulations

We used a base leave-on formulation (placebo) and an active leave-on formulation (treatment) consisting of the base formulation plus 3% *Epilobium fleischeri* extract (commercial product ALPAFLOR[®] ALP-SEBUM CB, DSM Nutritional Products). Base and active formulations are outlined in Table 1.

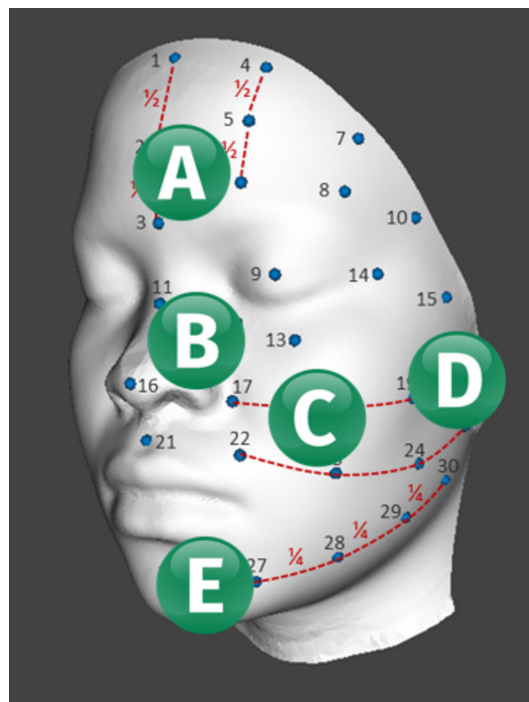


FIGURE 1 Five microbiome sampling areas on the face. A, forehead; B, nose; C, front cheek; D, lateral cheek; E, chin

Clinical study design

A placebo-controlled, single-blind and randomized clinical study was conducted at the Skin Test Institute in Neuchâtel, Switzerland. Study participants gave their informed consent to participate in the study, and the general principles of the Declaration of Helsinki guidelines were applied. The study was approved by the Reading Independent Ethics Committee, Woodley (UK). Adverse effects were recorded.

Twenty-three healthy Caucasian female volunteers aged between 18 and 40 (average age was 33.7 \pm 9.8 years) with hyperseborrheic skin were enrolled in the study. Volunteers were encouraged to refrain from any topical application on their face, apart from the test products, throughout the whole study duration. Acclimatization at the test institute was performed for about 30 min before any measurements or sampling were performed.

The study included a pre-conditioning phase lasting 5 days during which the study participants were refrained from using any sebo-regulating product on their face. This is to prevent any possible interference of previously used cosmetics with the skin biophysical measurements. During this time, period the study participants were provided with a gentle cleanser to be used for cleansing their face. The pre-conditioning phase was followed by the application phase, which lasted 4 weeks during which the products were applied on the face twice daily by the volunteers at home, previous training by the investigator, according to the randomization plan.

No product was applied on the face on the morning of the final skin assessments. A facial cloth soaked with lukewarm water was used to gently cleanse the skin. Ultimately, the skin was allowed to air-dry.

Porphyrins

Full face images were taken using the imaging system ColorFace® under UV light mode at 365 nm to visualize orange fluorescence caused by porphyrins. Fluorescence was quantified via digital image analysis. UV images were examined at baseline ($t = 0$), and only those volunteers showing orange fluorescence at baseline were selected for the quantification and comparison of the change between baseline and end of the treatment (4 weeks). The number of subjects with visible porphyrin fluorescence was five for the active group and nine for the placebo group. The region of interest for the porphyrin evaluation was limited to the nasolabial area in which most of the fluorescence was observed.

Non-inflammatory lesions

The evaluation of non-inflammatory lesions ('black heads' and 'white heads') was performed manually by a trained expert doing visual identification and counting on the ColorFace® high-resolution images directly. Inflammatory lesions (pustules and papules) were also assessed but were excluded from the final evaluation due to their not significant presence (0–3) in most of the volunteers. Hyperpigmentation, scars, nevi or other irregularities were excluded from the evaluation.

Skin microbiome sampling

Skin Microbiome of the stratum corneum was collected via swabbing by trained personnel who ensured that the same number of strokes and consistent pressure onto the skin was applied throughout the entire sampling procedure. Five sampling areas of 4 cm² each were defined on the face of each study participant. (1) forehead, (2) nose, (3) front cheek, (4) lateral cheek and (5) chin were chosen as sampling areas as representative of different facial sites with different skin features (Figure 1).

16S rRNA gene sequencing and predicted metagenomics

DNA extracted for qPCR was used for sequencing as previously described [46]. In brief, the V3-V4 hypervariable

region of the 16S rRNA gene was amplified using the 341F (5'-CCTACGGGNGGCWGCAG-3') and the 785R (5'-GACTACHVGGGTATCTAATCC-3') primers appended with Illumina adaptor sequences. The amplicons were sequenced on Illumina's MiSeq platform with paired-end 300 bp reads. Initial quality assessment was based on data passing the Illumina Chastity filtering. Subsequently, reads containing PhiX control signal were removed using an in-house filtering protocol. In addition, reads containing (partial) adapters were clipped (up to a minimum read length of 50 bp). The second quality assessment was based on the remaining reads using the FASTQC quality control tool version 0.11.5. Paired-end sequence reads were collapsed into so-called pseudoreads using sequence overlap with USEARCH version 9.2 [47]. These pseudoreads were collapsed into 97% OTUs. Classification of these pseudoreads was performed based on the results of alignment with SNAP version 1.0.23 [48] against the RDP database [49]. Taxonomic calls were based on a rank-specific identity threshold of Species 99%, Genus 97%, Family 95%, Order 90%, Class 85% and Phylum at 80%.

PICRUSt2 was used to reconstruct metagenomes [50]. Amplicon sequence variants (ASVs) were generated using DADA2 [50,51]. Forward and reverse read were trimmed to remove the first 10 bp, truncated to 250 bp length and with a maximum expected error of 1. Reads were merged with a maximum mismatch of 3 in the overlapping region. ASV's were fed through PICRUSt2 using default parameters to produce a predicted metagenome of E.C's and MetaCyc pathways [52].

Data filtering and analysis

As previously described in our former publication [46], for presentation of taxonomy and alpha/beta diversity metrics, singletons were removed from OTU level data and then collapsed based upon taxonomic classification. Alpha and beta diversity were evaluated using Shannon's metric and Bray–Curtis dissimilarity (scikit-bio v0.5.6).

Co-occurrence filtering methods were applied to establish shifts in the most common microbiota. A threshold of 75% was used to establish core microbiota across all participants to increase sensitivity [53]. We built the statistical model testing for differences between the study groups using Songbird [12]. Differentials were calculated according to the formula: Treatment[T.active] to indicate association of the features (OTUs) with samples obtained from the facial skin of participants using the active formulation, using placebo-valued samples as a reference. The reference value is used as the denominator in the log-fold change computation of differentials. In that way, for Treatment [T.active], OTUs with the

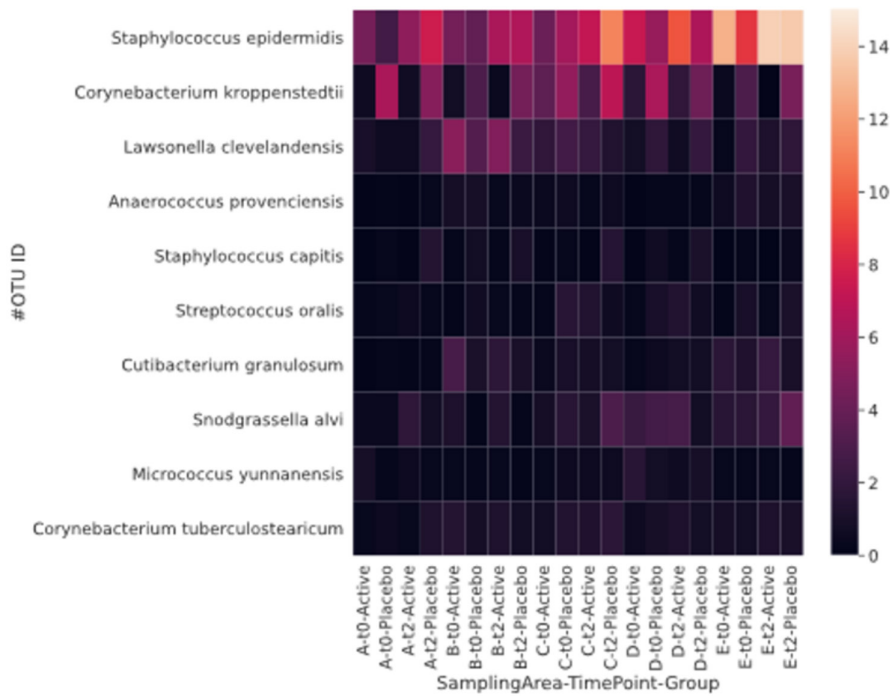


FIGURE 2 10 largest contributors to the relative abundance excluding *C. acnes* for all the different facial sites for the placebo/active groups at baseline (t0) and at 4 weeks (t2). A, forehead; B, nose; C, front cheek; D, lateral cheek; E, chin

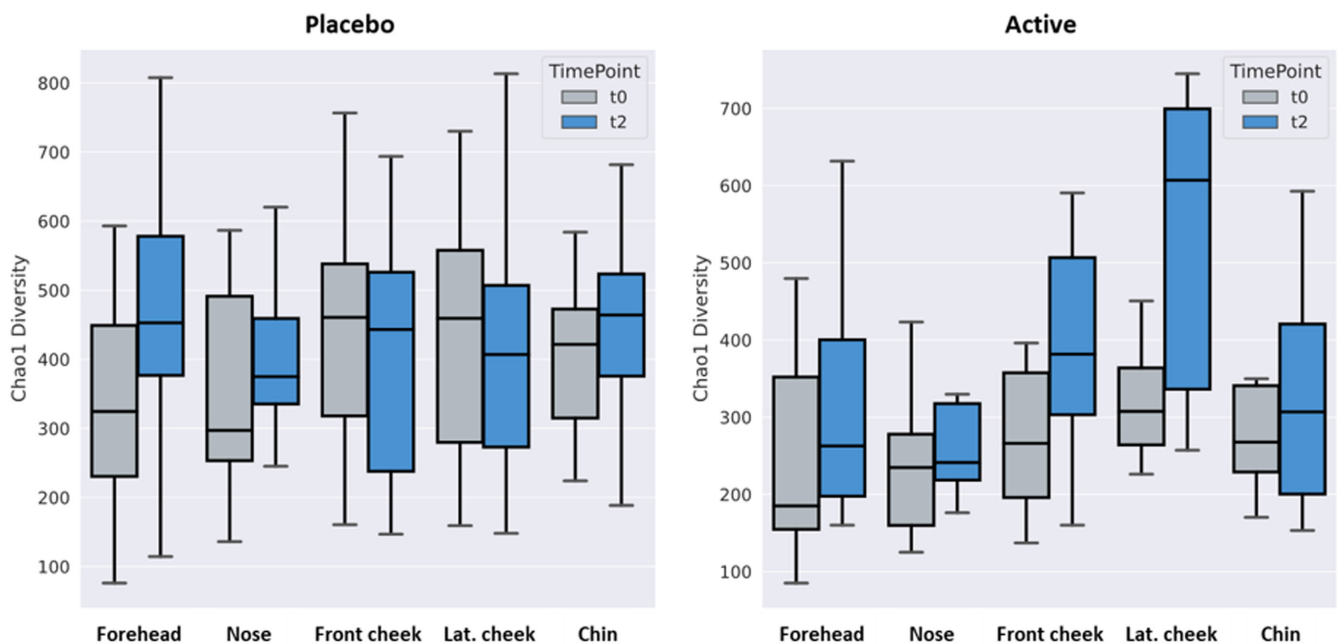


FIGURE 3 Alpha diversity analysis using Chao1 index for placebo and active groups. Data show Chao1 index at baseline (t0) and after 4 weeks of products application (t2). The only site to show significant changes in the Chao1 index was the lateral cheek of the active treatment group ($p = 0.006$)

most negative differential ranking values will be more associated with placebo-valued samples, whereas the features with the most positive differential ranking values will be more associated with active-valued samples. When analysing Songbird differentials, the top 10% of features associated with the active treatment were selected and then evaluated. The bottom 10% of features have been used as reference frames to infer changes in the microbial composition.

Facial colour mapping

Colour maps were generated by combining the mean 3D images and the median values of bacteria pairs' log-ratios for each study group. An algorithm was developed which automatically detects skin pixels and interpolates a value for each of them superimposing the log-ratio data on the images. This results in full and continuous 3D colour maps. A gradient of blue colour was assigned to

indicate higher log-ratio values ($0 < \log\text{-ratio} < 2$), whereas a gradient of red colour was assigned to indicate low and negative log-ratio values ($-2 < \log\text{-ratio} < 0$). The changes projected onto a 3D face allow for the identification and visualization of the facial sites in which the microbial shift occurred.

RESULTS

Relative abundance and diversity

As expected, *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*, which is known to be present on facial skin and on the scalp, and *Micrococcus yunnanensis* (Figure 2). Bray–Curtis dissimilarity of the samples showed intermixed samples based on family-level comparison (Figure S1). While there was a broader dispersion of Placebo samples, the treatment group and placebo were not distinctly different.

Microbial alpha diversity before and after product application has been assessed via the Chao1 index (Figure 3). Major differences were observed between the two groups.

Being the Chao 1 index an abundance-based estimator of species richness, it appeared evident that the group applying the active formulation resulted having an overall increased species richness after 4 weeks of product application in all the facial sites of interest as compared to baseline (t0).

In contrast, this increase in species richness was not observed in the group applying the placebo formulation. However, it is worth to consider that the placebo group resulted entering the clinical study (t0) with a with higher Chao 1 index values as compared to the active group. Shannon index showed not to be particularly affected by any of the products (Figure S2).

Reference frames and differential ranking

We used Songbird to calculate differentials, meaning logarithmic fold changes of taxa abundances between two conditions (placebo and active) as well as the ‘reference frames’ approach to infer compositional changes resulting from the application of the active formulation [12]. We built the statistical model testing for differences between the study groups; therefore, Songbird calculated differentials based on the formula: $\text{Treatment}[\text{T.active}]$. The resulting differentials indicate the association with samples obtained from the facial skin of participants using the active formulation using placebo-valued samples as a reference. The reference value is used as the denominator in the log-fold

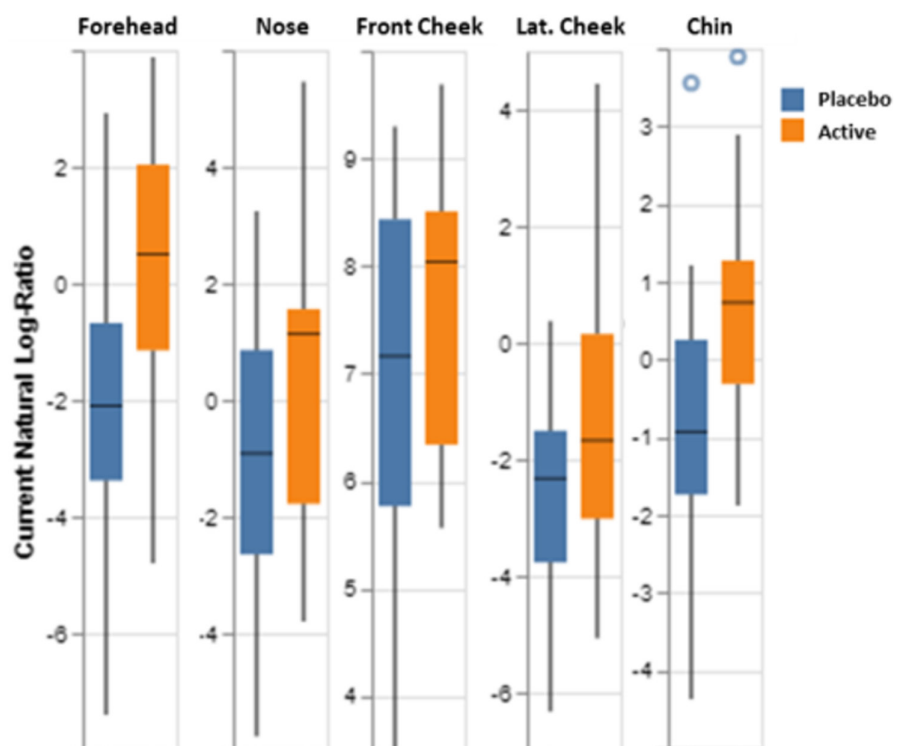


FIGURE 4 Top 10% OTUs in the differential rankings produced by songbird and visualized via Qurro for the placebo and active groups after the 4 weeks-long treatment phase. The box plots represent differences in the log-ratios of the top 10% taxa between the groups after 4 weeks of products application, with the bottom 10% OTUs taken as ‘reference frames’

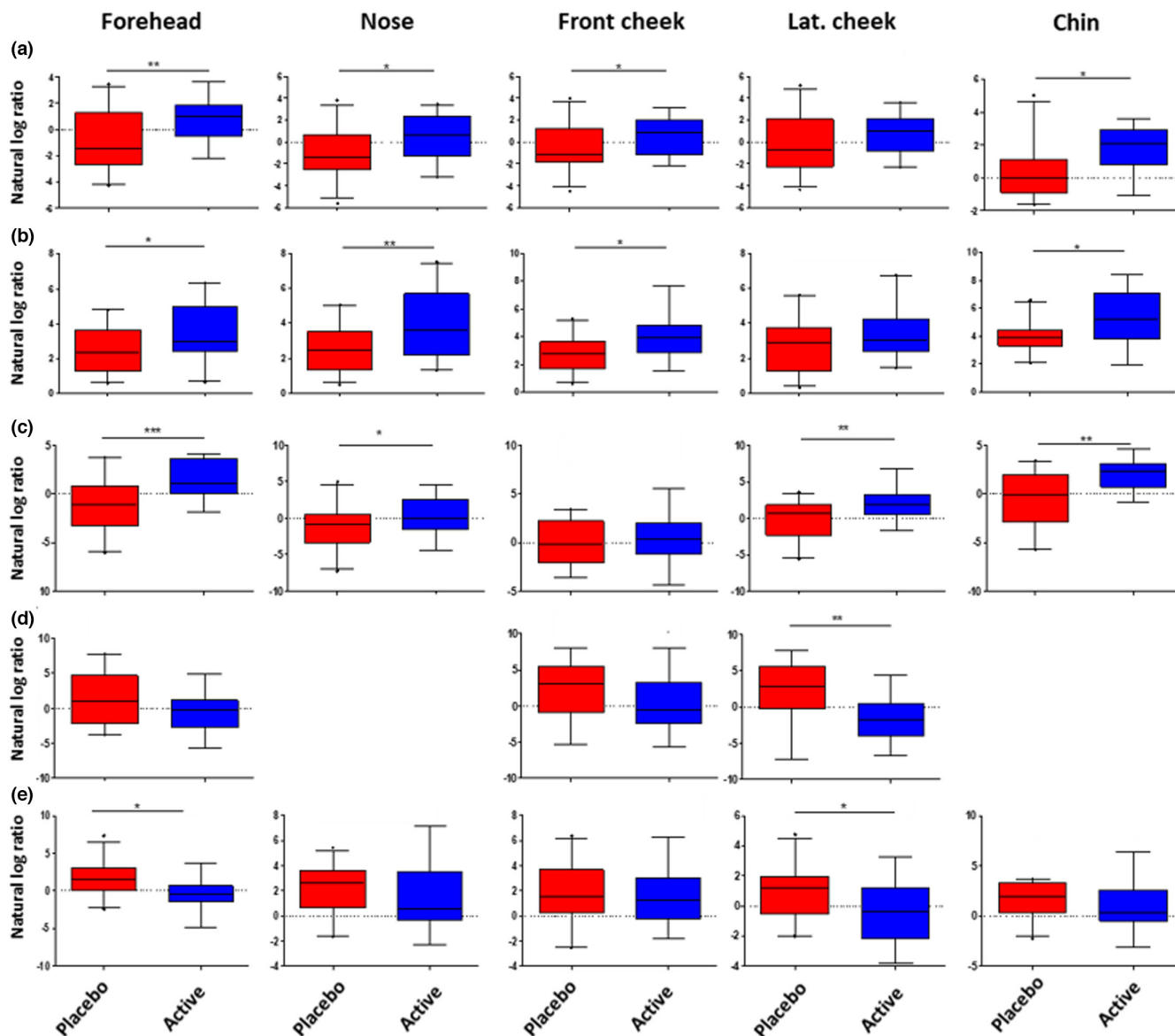


FIGURE 5 Log-ratio of several taxa identified from the differential ranking analysis. Box plots illustrating the natural log-ratio of (a) *S. hominis/S. capitis*; (b) *S. epidermidis/S. capitis*; (c) *M. yunnanensis/S. capitis*; (d) *C. kroppenstedtii/M. yunnanensis*; (e) *C. tuberculostearicum/M. yunnanensis*; across the placebo and active groups. Statistical significance has been calculated via Welch's *t*-test (* $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$)

change computation of differentials. In that way, for Treatment[T.active], the features (microorganisms) with the most negative differential ranking values will be more associated with placebo-valued samples, whereas the features with the most positive differential ranking values will be more associated with active-valued samples. 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 (Figure 4).

An increase in the natural log-ratio was observed in all facial sites in the group applying the active formulation as compared to placebo. Therefore, the data showed a clear

shift of the core skin microbiota, which was associated with the presence of the *Epilobium fleischeri* extract in the product. By looking at the differential ranking graphs, we could identify key taxa, which were positively associated with the active formulation and 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. In contrast, *Micrococcus yunnanensis* often ranked higher than most of the other community members. We therefore looked at log-ratios to examine shifts in the facial skin microbial composition (Figure 5). These were also made visible via a facial colour mapping approach (Figure 6).

FIGURE 6 Visualization of log-ratio between *S. epidermidis*/*S. capitis* via facial colour mapping. The colour maps show log-ratio increase in *S. epidermidis*/*S. capitis* after 4-week treatment with the placebo (left) and with the product (right). Colour code (−2 to 2) is shown on the scale on the right-hand column. (A, B, C, D, E were the facial sampling areas)

Log-ratio *S.epidermidis*/*S.capitis* after 4 weeks of product application

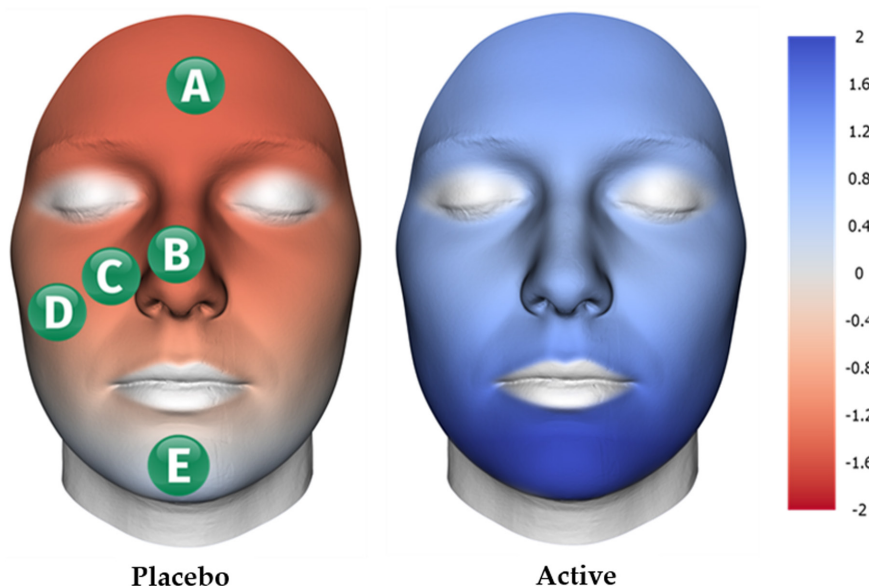


TABLE 2 Songbird output values for pathways related to N-acetylneuraminate degradation, mandelate degradation, hexitol degradation and tryptophan degradation

	MetaCyc Pathway	Time	Site [T.B]	Site [T.C]	Site [T.D]	Site [T.E]	Treatment [T.active]
N- acetylneuraminate degradation	GLCMANNANAUT-PWY	0.036	0.997	0.743	0.148	0.491	−0.627
	P441-PWY	−0.048	0.729	0.609	0.195	0.468	−0.480
Mandelate degradation	PWY-1501	0.549	−2.535	−0.815	−2.273	0.258	−2.646
	PWY-6957	0.755	−3.295	−0.578	−2.154	0.271	−2.704
Hexitol Degradation	HEXITOLDEGSUPER-PWY	−0.173	0.406	0.231	0.033	0.153	−0.440
	P461-PWY	−0.239	0.191	0.041	−0.026	−0.097	−0.476
Tryptophan	NADSYN-PWY	−0.0142	−1.299	−0.551	−0.478	0.117	1.533
	PWY-5651	−0.139	−1.381	−0.584	−0.549	0.262	1.677

As the extract containing the ellagitannin oenoethin B within the skin active is expected to be inhibitory, we focused on the negatively associated pathways produced from Songbird (Table 2). The negative Songbird associations of reconstructed pathways from PICRUSt2 contained several MetaCyc pathways referring to n-acetylneuraminate degradation (GLCMANNANAUT-PWY and P441-PWY), mandelate degradation (PWY-1501 and PWY-6957), hexitol fermentation (P461-PWY and HEXITOLDEGSUPER-PWY) and tryptophan degradation (NADSYN-PWY and PWY-5651). N-acetylneuraminate degradation was positively associated with all facial sampling sites except the forehead, as did hexitol degradation. Mandelate degradation had a negative association with all facial sites except for the forehead. The mandelate and N-acetylneuraminate degradation pathways were mixed in their association with time, whereas hexitol degradation showed a negative association with time. Tryptophan

degradation is also connected with NAD biosynthesis and was only positively associated with the chin.

Porphyrins and non-inflammatory lesions assessment

We 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 7). 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.

The presence of non-inflammatory lesions, such as white and black heads, was also assessed in the two different cohorts. Following the 28 days of product application,

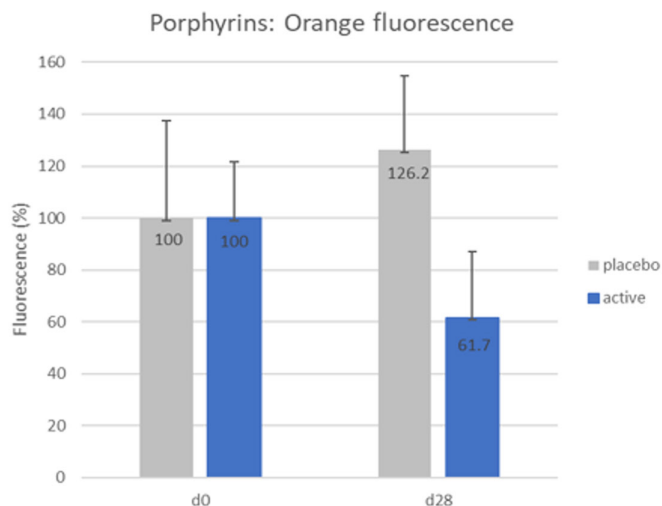
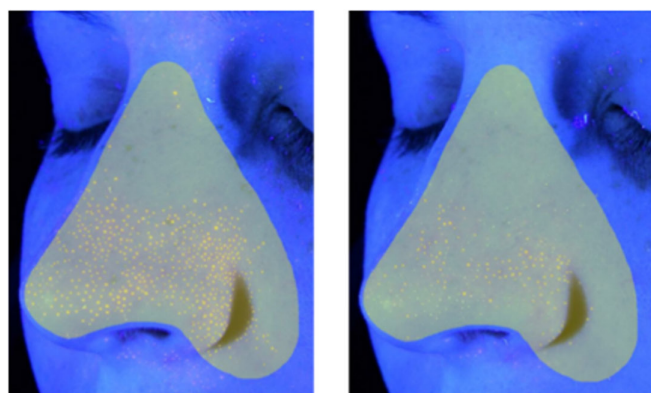


FIGURE 7 Porphyrins assessment. Left, detail of volunteer n.6 (27 y.o.), left profile, with ROI and segmented porphyrins at D0 and D28. Right, fluorescence quantification indicating a significant reduction in the active group after 28 days

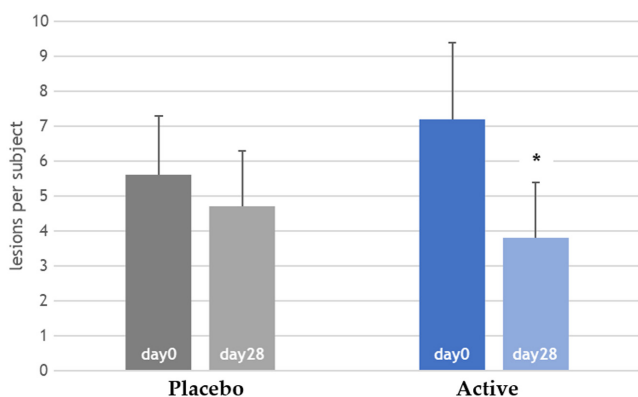


FIGURE 8 Number of non-inflammatory lesions identified per subject. Shown are mean values +SEM. * $p < 0.01$ (paired t -test)

the number of lesions per subject was significantly reduced ($p < 0.01$) in the active group. The magnitude of the effect was of 47% less non-inflammatory lesions in the active group, whereas in the placebo group, only a slight but not significant reduction was observed (Figure 8).

DISCUSSION

Excess sebum production on facial skin represents a concern for many people as it results in a shiny and greasy skin appearance. Consumers are therefore constantly looking at effective cosmetic skin care solutions, which could help in reducing the unwanted overproduction of sebum [13].

In this study, we looked at the effects of an *Epilobium fleischeri* extract on facial skin in addition to its already known strong sebum-regulating properties. Our attention was focused on the improvement of the overall skin

phenotype and how this is reflected in the microbiota composition of different facial sites. We intentionally enrolled study participants with oily skin and excess sebum production in order to have a population with acne-prone skin represented and to assess to which extent the active formulation could help improving this common skin phenotype and to be able to map microbial changes in response to the product application.

Taxonomic profiling showed *C. acnes* being the most abundant species by far across all the investigated facial sites followed by *S. epidermidis*. The abundance of *C. acnes* is known to vary with age and sex in healthy individuals and to be characterized by a steep increase during puberty, remaining stable until old age, then decrease again in association with the reduction in sebum production of aged skin [14]. We found that the *C. acnes* was not particularly associated with neither the active nor the placebo formulation use. *C. acnes* is a renowned lipophilic skin commensal with beneficial protective effect in healthy skin. By metabolizing sebum into free fatty acids, the bacterium can prevent the colonization of skin by pathogenic microbes and inhibit biofilm formation [15, 16]. *C. acnes* abundance and strain diversity are reduced in certain skin diseases, including acne, atopic dermatitis and psoriasis [17–19]. In particular, specific subgroups (phylogroup IA₁) are associated with acne and are thought to play an important role in the pathogenesis of the disease. Such strains are known to produce larger levels of virulence factors, such as porphyrins than other healthy phylotypes [20–22]. Interestingly, here, we could observe a significant decrease in porphyrins on the skin of volunteers which were applying the product containing the *Epilobium fleischeri* extract. 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, even though the overall abundance of *C. acnes* species has not been particularly modulated as compared to other taxa.

In addition to that, we could identify microbial shifts and infer microbiota compositional changes resulting from the twice-daily and 4-week long application of the active formulation containing the *Epilobium fleischeri* extract. Certain microbiota species, such as *Staphylococcus hominis*, *Staphylococcus epidermidis* and *Micrococcus yunnanensis*, resulted positively associated with the use of the active product. These three bacterial taxa are known skin commensals providing several beneficial properties for healthy-looking skin, such as the secretion of antimicrobial peptides by *S. epidermidis* and *S. hominis*. [23–25] *M. yunnanensis* belongs to the *Micrococcus luteus* group as both are phenotypically and genotypically closely related [26]. This species is known to be able to withstand high levels of UV radiation and to produce carotenoids providing important antioxidant and antibacterial activities [27, 28]. These bacteria resulted to be significantly enriched in the final microbiota composition after 4 weeks of active product use as compared to the placebo. *S. capitis*, a traditionally considered commensal, is among the many coagulase-negative staphylococci (CoNS) species which are now recognized as opportunistic human pathogens. In recent years, the genome of *S. capitis* isolates has been studied in order to identify genes which are predicted to be important for *S. capitis* virulence [29]. *C. kroppenstedtii* is a Gram-positive lipophilic bacterium, which is known to be enriched on the skin of rosacea affected patients and increased levels are typically observed in clinical cases of skin redness [30–32]. Despite being a commonly observed commensal species, several studies associated *C. tuberculostearicum* with disease state, including inflammatory breast disease, sinusitis and surgical site infection [33–35]. Furthermore, it has been proposed that *C. tuberculostearicum* could be able to initiate and perpetuate chronic inflammatory skin diseases via activation of the canonical NF- κ B pathway [36]. 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, that is sebaceous area vs dry areas (e.g., forehead vs lateral cheek). Four-week-long topical application of a natural *Epilobium fleischeri* extract rich in oenothien B did not impact the natural skin microbial diversity, but increased microbial richness, as shown by the Chao1 index. It was even more interesting to observe the significant microbiota modulating properties of the extract 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*.

Analysing the pathways predicted from PICRUST2 using Songbird, three trends were apparent: N-acetylneuraminate degradation, mandelate degradation and hexitol degradation were negatively associated with the active treatment. N-acetylneuraminate degradation, otherwise known by the enzyme neuraminidase, has been implicated in viral diseases like influenza and in bacterial respiratory infections [37]. Literature also suggests that these bacterial neuraminidases may be implicated in seborrheic eczema [38]. Given the significance of neuraminidases, research into inhibition of these enzymes has produced several ellagitannins [39], it suggests the ellagitannin in our active treatment can act in a similar manner but follow-up studies should confirm. The inhibitory effect may also be impacting mandelate degradation. As mandelic acid has been reported to be beneficial in treating acne and hyperpigmentation, the degradation could have adverse effects on the skin quality such as viscoelasticity [40]. Mandelic acid may also help reverse the skin ageing process [41]. Wójcik et al. also reported increased sebum secretion in the U-zone, but the mandelic peel had no effect in the T-zone [41]. The treatment active may be acting synergistically with mandelic acid by preventing the degradation of this valuable skin compound. Lastly, the treatment active appears to have an additional inhibitory effect on hexitol degradation. While several hexitols could be the target of such degradation, mannitol has the most relevance to skincare [42]. Taieb et al. suggest that mannitol in combination with hyaluronic acid can improve skin hydration and elasticity. Additionally, mannitol fermentation by *Staphylococcus aureus* is required to provide protection from natural skin antimicrobial activity [43]. While the mechanism for inhibition is unknown, preventing degradation again may contribute to synergistic effects of applied mannitol by preventing the microbial removal of this valuable compound. The treatment active may also have implications in reducing *S. aureus* by the circuitous manipulation of its ability to metabolize mannitol and allow it to succumb to the natural antimicrobial activity of the skin. Lastly, tryptophan degradation appears to be feeding microbial metabolism as evidenced by the connection to NAD biosynthesis. Increased tryptophan degradation is also representative of healthy skin when compared to subjects with atopic dermatitis and lesioned skin from with hidradenitis suppurativa [44, 45]. Geunin-Macé et al. suggest that the metabolism of tryptophan to indoles may reduce inflammation and this may be connected to the effect we see with reduced porphyrins in the active treatment. The tryptophan degradation effect, in

addition to the inhibition of the other pathways, suggests that the active treatment is actively manipulating the microbial community at the metabolic level in a manner that appears to promote healthy skin.

AUTHOR CONTRIBUTIONS

D.I. conceptualized and designed the clinical efficacy study. J.C. applied ‘reference frames’ methodology and software programming for the next-generation sequencing data analysis. R.S., J.C. and D.I. contributed to the interpretation and discussion of the microbiome data. All authors contributed to the writing of the manuscript, have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

All authors are employees of DSM and receive regular salaries from the company. The authors declare no other conflicts of interest exist.

DATA AVAILABILITY STATEMENT

The data will be available under NCBI bioproject PRJNA778761.

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Additional supporting information may be found in the online version of the article at the publisher's website.

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