

Visualization of changes of the facial sebum maps and microbiome maps after treatment with sebum inhibitor formulation



Dominik Imfeld,¹ Zheng Zhou,² Miao Guo,² Mathias Gempeler¹

¹ DSM Nutritional Products Ltd., Basel, Switzerland

² Mageline Biology Tech. Co. Ltd., Wuhan City, China



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Abstract

We selected 23 human female pre-menopausal volunteers with elevated sebum level on forehead to perform a study on sebum modulation and in addition measuring microbiome profiles. In analogy to the moisture mapping, we determined the sebum level by Sebumeter on 30 sites in the face. Volunteers have been treated during four weeks with a formulation including a known plant-based sebum inhibitor made from *Epilobium fleischeri* extract to show the effects on the sebum maps before and after the treatment in comparison to baseline and to a placebo formulation without plant extract.

Visualization of sebum maps showed that the sebum distribution in the face is more complex than the well known "T-zone" with also significant sebum levels and gradients on the cheek area and further down to the jawline. For the volunteers starting with high (> 120 units) sebum level we observed a significant reduction of 26% on the cheek area and 16% reduction on the forehead after 4 weeks of treatment.

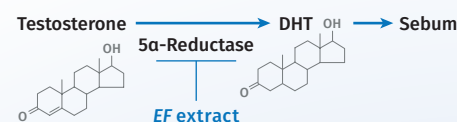
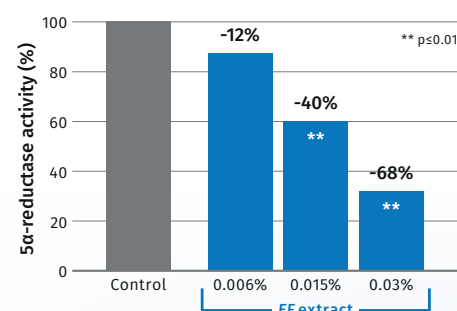
At the same time, we collected microbiome samples from the face by the swabbing approach and analysis with 16S-rRNA sequence profiling on five sites, namely on the frontal-, nasal-, infraorbital-, infratemporal-, and chin-area to correlate the microbiome composition to the sebum level on the one hand and also to observe the changes in the microbiome composition caused by the treatment of the *Epilobium fleischeri* extract.

In summary with the current study we visualized the facial sebum map and the differential effect on the site-specific distribution that was achieved after treatment with a sebum inhibitor formulation based on *Epilobium fleischeri* extract. We also analyzed the changes in the microbiome composition specific to the facial position, in correlation to the sebum level and also by the treatment with *Epilobium fleischeri* extract.

Epilobium fleischeri (EF) Extract for Sebum Regulation

Some key facts about the EF extract:

- Is growing in the Alps as unique glacier willowherb species, *Epilobium fleischeri*, now organically cultivated for commercial purposes
- Extract contains high levels of oenothien B
- Has been shown *in vitro* to be an inhibitor of 5- α -reductase which is direct biological target to decrease sebum production
- Also *in vitro* shown as inhibitor of pro-inflammatory markers Interleukin 8 and IL1 α inhibition
- Was a promising candidate for sebum mapping because previously shown *in vivo* to inhibit sebum production on facial skin

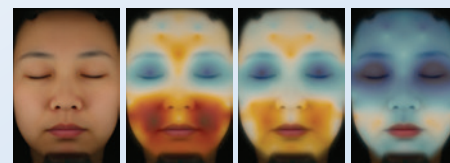


Significant dose-dependent inhibition of 5 α -reductase activity up to 68% versus control with 0.03% *Epilobium fleischeri* extract in human skin fibroblasts.

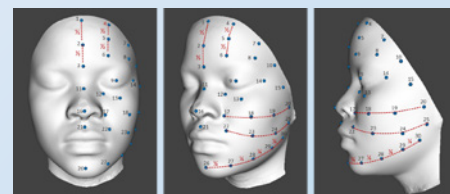
Sebum Mapping: Continuous visualization of sebum level for the face

We have previously shown and applied this approach for moisture mapping¹

- Sebum level measured by Sebumeter
- Data collection on 30 test sites in the (hemi-) face (same setup as for the moisture maps)
- Mapping algorithm by interpolation between measured values in analogy to the moisture map method
- Color bar defined for sebum level 20 – 200 $\mu\text{g}/\text{cm}^2$ (for the first baseline map)



Example of moisture mapping images generated for hydration study



Showing positions of data collection on 30 test sites in the (hemi-) face (same setup as for the moisture maps)

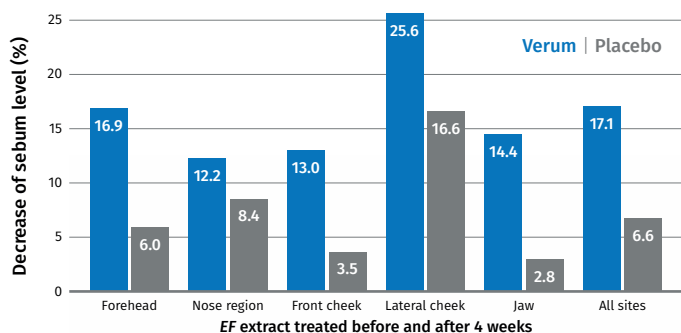
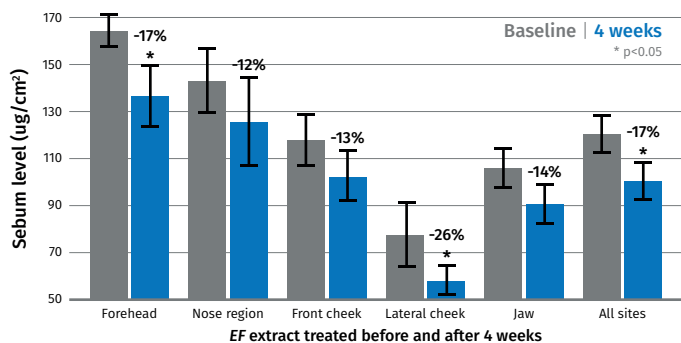


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Results of *in vivo* facial sebum regulation by EF extract

Strong decrease of sebum level after 4 weeks across all parts of the face



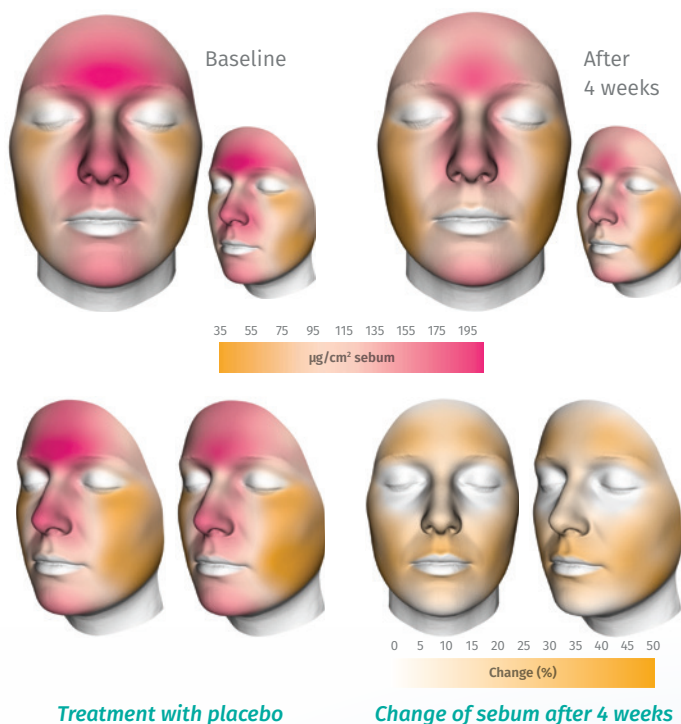
EF extract treatment rebalanced the sebum to normal levels with significant reduction on the full face (17%) and especially on the forehead (17%) and on lateral cheek (26%) after 4 weeks and better than placebo in any facial sites

In vivo study setup:

- Volunteers with > 120ug/cm² Sebum on forehead were recruited, the verum group contained 10 volunteers, the placebo group contained 13 volunteers
- Age range 33.7 ± 9.8 years (min 19.5y, max 51.1y)
- Sebum data were recorded at baseline and after 4 weeks of treatment by means of biometric measurements (Sebumeter® SM 810, Courage & Khazaka). Microbiome samples were collected by swabbing at baseline and after 4 weeks
- EF extract and placebo was applied 2x per day for 4 weeks
 EF extract formulation used: ACRYLATES/C10-30 ALKYL ACRYLATE CROSSPOLYMER 0.3%, (PHENOXYETHANOL, ETHYLHEXYLGLYCERIN) 1.0%, (GLYCERIN, AQUA, CITRIC ACID, POTASSIUM SORBATE, EPILOBIUM FLEISCHERI FLOWER / LEAF / STEM EXTRACT)* 3%, AQUA added to 100%
- The study protocol and the informed consent form were reviewed and approved by the Readings Independent Ethics Committee Woodley UK, prior to the initiation of the study. The benefits and risks of participation in the study were explained to each subject prior to entering the study and then all subjects were given written informed consent

Sebum mapping

Before and after 4 weeks treatment with EF extract



Microbiome analysis:

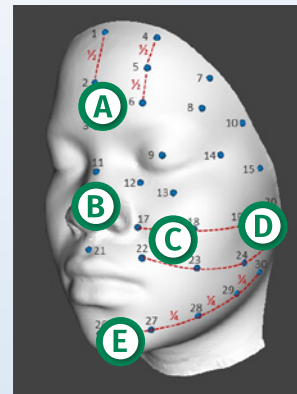
Correlation of Sebum level and EF extract treatment to microbiome composition

Five Microbiome sampling areas:

- A Forehead (1, 2, 3, 4, 5, 6)
- B Nose wing (11, 16)
- C Front cheek (13, 17, 18, 22, 23)
- D Lateral cheek (19, 20, 24, 25)
- E Jaw (26, 27)

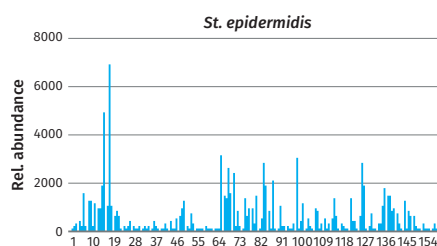
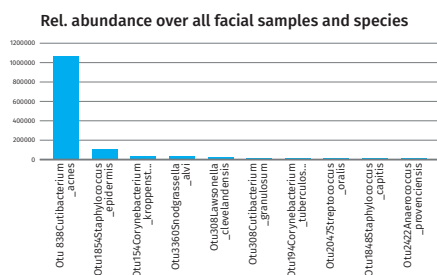
30 Sebum sampling sites: (no. 1 to 30)

Average sebum level from several data points were used to correlate with the microbiome sample area

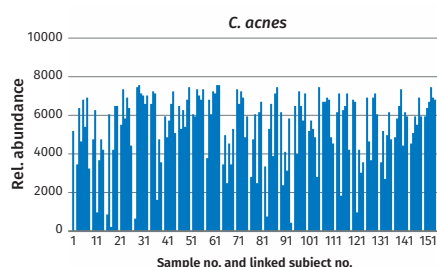


Relative Abundance Data

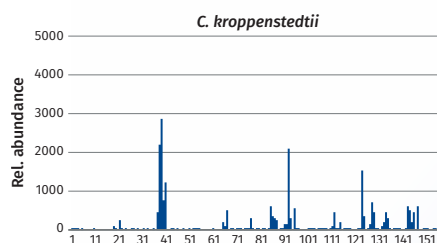
From 16S rRNA sequencing at t=0



Staphylococcus epidermidis second most abundant, but less homogeneous distribution



Cutibacterium acnes by far most abundant in the face with homogeneous occurrence



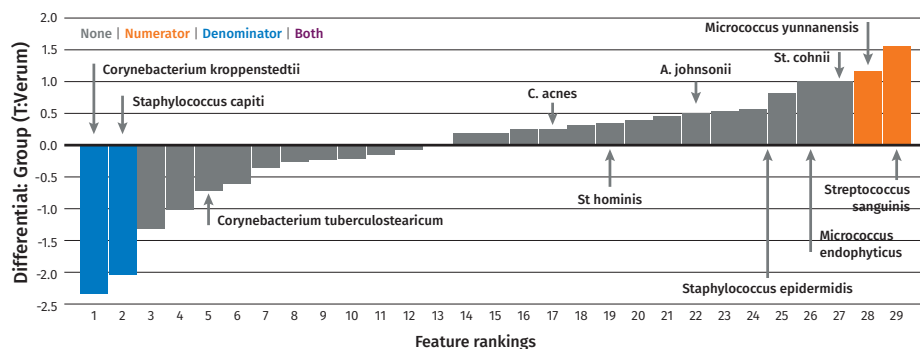
Corynebacterium kroppenstedtii also abundant but few subjects with high load vs others were not infected



Flowers of *Epilobium fleischeri*

Visualizing microbiome composition modulation using the tool Qurro²

Qurro is the software to show feature rankings and log-ratios

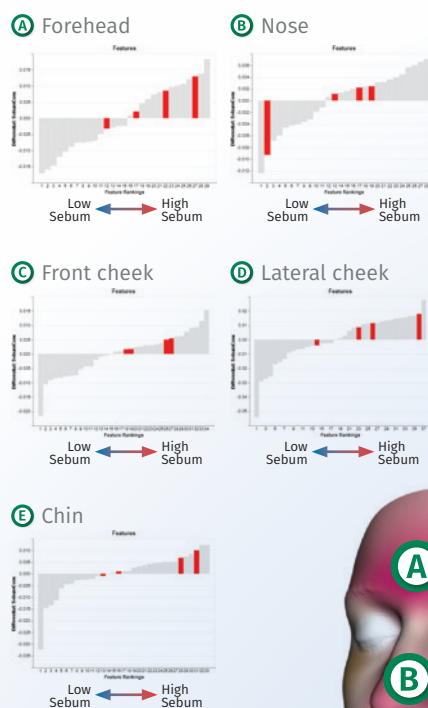


Rank Plot showing differentials for forehead by EF treatment $\log \left(\frac{\text{Verum}}{\text{Placebo}} \right)$

Propionibacterium genus members are associated with high sebum content

Red color highlighted = *Propionibacterium*

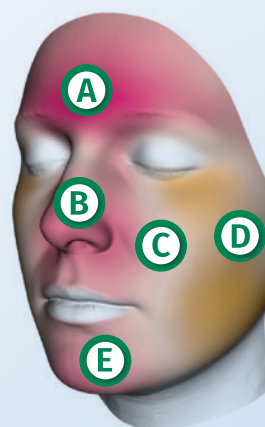
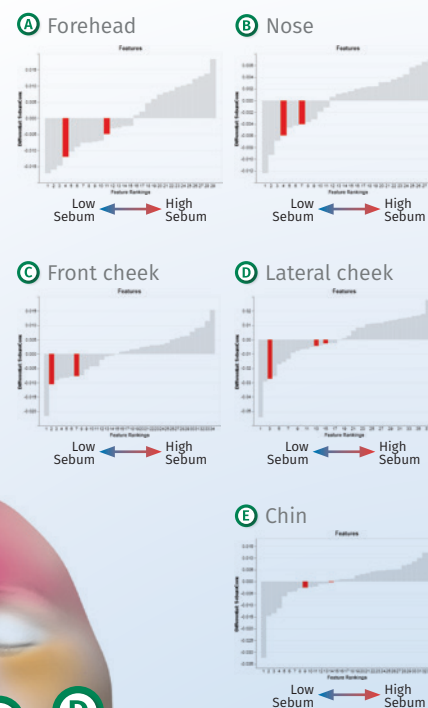
Log-Ratio classification: None | Numerator



Micrococcus genus members are associated with low sebum content

Red color highlighted = *Micrococcus*

Log-Ratio classification: None | Numerator

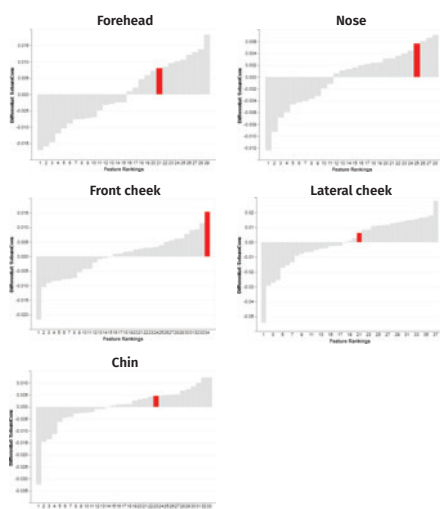


Staphylococcus capitis is down by EF extract treatment on all facial sites

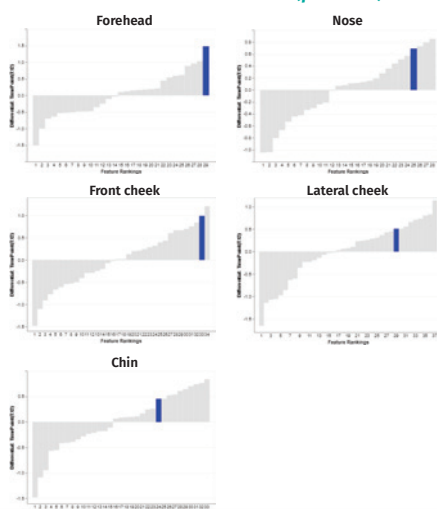
From 16S rRNA sequencing at t=0

Staphylococcus capitis is a coagulase-negative species (CoNS) of *Staphylococcus*. It is part of the normal flora of the skin of the human scalp, face, neck, scrotum, and ears and has been associated with prosthetic valve endocarditis. *S. capitis* is presumed having role in acne disease progression.

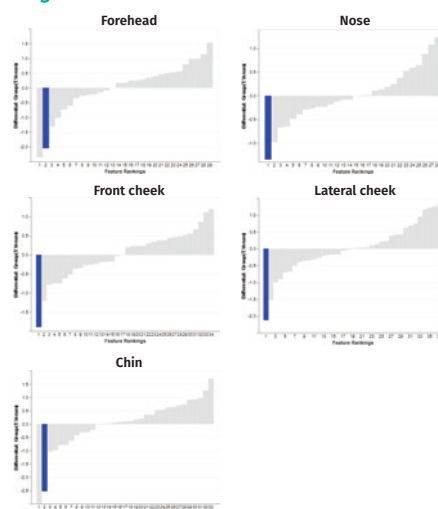
Positive correlation with sebum content



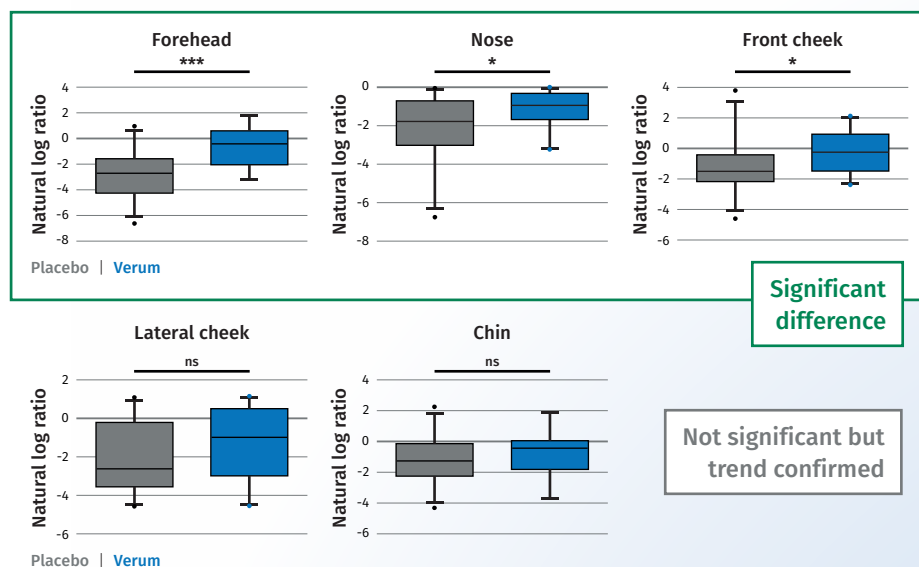
Positive correlation with time (placebo)



Negative correlation with EF extract treatment



The log-ratio *S. hominis*/~10% bottom ranked microbes significantly increases with EF extract treatment



Conclusions

Sebum mapping: It demonstrates a T-zone like but complex pattern of facial sebum distribution as expected with the highest sebum levels on the forehead but also with a strong sebum concentration gradient from nose and front cheek area to the lateral cheek area. The treatment with EF extract showed sebum reduction on all facial areas compared to baseline and to placebo.

Microbiome mapping: We defined 5 microbiome sampling areas with either high or low sebum levels to correlate the sebum level with the composition of the microbiome. The genus of *Propionibacterium* has been associated to facial sites with high sebum level whereas members of the genus *Micrococcus* was associated with low sebum areas. The treatment with EF extract showed a reduction of *St. capitis* on all facial areas but preferred the abundance of *St. hominis*. More microbiome analysis is ongoing from this study.

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References

- Voegel et al. International Journal of Cosmetic Science, 2019, 41, 411–424
- Fedarko et al. NAR Genomics and Bioinformatics, 2020, Vol. 2, No. 2