The effect of two different creams on the skin microbiome composition, *c.acnes* in particular, and skin appearance

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A balanced skin microbiome is important for maintaining a healthy skin. When this balance is disrupted, skin diseases may develop. Traditionally these diseases are treated by applying atopic antibiotics. Drawbacks of using antibiotics are the selection of antibiotic resistant microbes and damage to commensal bacteria. In a small, controlled study we found no significant effect of both organic acids and fructo-oligosaccharides (FOS) as microbiome altering compounds in skin cream on the relative prevalence of *c.acnes* as well as the overall microbiome diversity. Nor did we find an effect on the appearance of the skin by using these creams. This work provides an important insight in how full 16s rRNA gene long read Nanopore sequencing and image analysis can be combined to study the effect of skincare products on the microbiome.

Introduction

The dry and acidic wasteland that we call our skin, is home to a variety of micro-organisms, with the majority being harmless commensals. However, certain microorganisms can contribute to the development of skin diseases such as acne vulgaris, psoriasis, atopic dermatitis, and chronic wounds. Under normal conditions the latter are commensals, but when the balance of the skin microbiome is disrupted, they may become opportunistic pathogens (Chen et al., 2018; Grice & Segre, 2011; Ito & Amagai, 2022). Acne vulgaris, is a common condition that affects an estimated 90% of the adult population at some point in their life (Gebauer, 2017; Heng & Chew, 2020; Tan & Bhate, 2015). The bacteria C*utibacterium acnes* are implicated to be involved in the development of acne, specifically certain phylotypes producing high concentrations of polyphyrins (Mayslich et al., 2021). Traditional treatment methods for microbe-caused skin diseases include topical anti-biotics (Yang et al., 2022), which have drawbacks such as the selection of antimicrobial-resistant micro-organisms and the collateral damage to commensals (Jo et al., 2021).

Recently skin-care companies have started promoting prebiotic creams, soaps and shampoos as a natural way to bring balance to the skin microbiome and decrease 'skin problems' (*Oolaboo Morning Dew Prebiotic Face Cream – Oolabooshop*, n.d.). (*Prebiotica: Een Must Voor de Gevoelige Huid! | Biodermal*, n.d.) Prebiotics are defined as substances that promote the growth of host micro-organisms that can provide a health benefit. Common prebiotic compounds include fructans such as inulin, oligofructose and fructo-oligosaccharides (FOS). Other well-known substances include galacto-oligosaccharides (GOS), arabinose, raffinose, soy oligosaccharides (SOS) and xylo-oligosaccharides (XOS). FOS being the most accessible and showing promising results (Bustamante et al., 2020; le Bourgot et al., 2022). Another promising treatment for acne is that of applying chemical peeling to the skin where acne is present, exfoliating the stratum corneum, consequently reducing the acne lesions. Salicylic acid among other compounds, like glycolic acid have been shown to have this effect on the skin (Arif, 2015; Lu et al., 2019; Sarkar et al., 2019).

Prebiotic creams could revolutionize the way skin diseases are treated. Instead of eradicating the pathogenic micro-organisms with anti-biotics, prebiotics that target the growth of commensal micro-organisms could provide a viable alternative to prevent dysbiosis and treat skin diseases. However, in contrast to the claims made by the skin care companies, the impact of prebiotic substances on the balance of the skin microbiome is not well understood. The topical application of prebiotics has not been investigated as extensively as their effect after ingestion on the gut microbiome (Bustamante et al., 2020). Previous studies have shown that even common ingredients in skin care products can have an impact on the skin's microbiome (Dobler et al., 2019; Murphy et al., 2021; Pinto et al., 2021). Further investigation is needed to determine if prebiotics can decrease the relative abundance of *c.acnes specifically* and preferably the subtypes associated with acne. Additionally, it is important to investigate whether the use of prebiotics leads to an increase in diversity of the skin microbiome, as suggested by the skincare companies.

Genetic techniques would be favourable to analyse microbiome species composition. The researcher's toolbox today consists of metagenomic gene-marker analysis such as 16S rRNA, shotgun metagenomics, metabolomics, metaproteomics and metatranscriptomics. Currently the advantages of gene-marker analysis in terms of costs and analysis consensus outweigh the benefits of shotgun metagenomics (Galloway-Peña & Hanson, 2021). The bacterial 16S rRNA gene consists of 9 variable regions (V1-9) separated by evolutionary conserved

regions. Long read sequencing of a whole 16S rRNA gene amplicon using Nanopore MinION technology could improve the identification of operational taxonomic units (OTUs) (Jain et al., 2016). This is advantageous compared to partial 16S data provided by short-read sequencing methods, but the downside would be the lower throughput and accuracy (Johnson et al., 2019; Matsuo et al., 2021).

The metagenomic approach enables researchers to analyse large numbers of microorganisms in environmental samples. Previous studies show that the 16S rRNA sequencing is a reliable method for skin microbiome analysis and is widely used in skin microbiome studies (Byrd et al., 2018; Kong et al., 2017). Due to the popularity of 16S rRNA sequencing in studies, several classification tools have been developed that can classify bacteria at multiple taxonomic levels. Nanopore Technologies provides several workflows of 16S sequencing data on the EPI2ME cloud service (Oxford Nanopore Technologies). Classification tools for this type of data have been compared and benchmarked, and it has been found that using Kraken2 in combination with Bracken enables accurate classification of bacterial reads at the species level (Almeida et al., 2018; Govender & Eyre, 2022). Furthermore, a metagenomics workflow using 16srRNA gene amplification to analyse bacterial compositions of the skin microbiome allows diversity indexes such as the Shannon-index and (inverse) Simpson- index to be calculated (Shannon, n.d.; Simpson, 1949).

In this study, we aim to investigate the effect of a prebiotic cream on the relative abundance of the C.acnes genus of the gram-negative Cutibacteriaceae (Propionibacteriaceae) family. Furthermore, we want to investigate the effect of two different prebiotic ingredients Fructooligosaccharides (FOS), and a mix of exfoliating carbo hydric acids (Salicylic acid and Glycolic acid) on the composition of the skin microbiome. Apart from these ingredients, the base cream should have a minimum effect on the skin microbiome. To measure the isolated effect of both creams the study is conducted using a control cream without the active ingredients. Image analysis is used to determine whether the skin develops irregularities. Such as inflammation seen as an effect on redness of the skin or the development of spots.

Material and methods

Cream and trial

6 individuals were randomly assigned to two groups in a double-blind study. Each participant received a cream containing prebiotics and a control cream without prebiotics. Both creams were used twice daily for two and a half weeks on both sides of the neck, just below the ears. 6 individuals were randomly assigned to two groups in a double-blind study. Each participant received a cream containing prebiotics and a control cream without prebiotics. Both creams were used twice daily for two and a half weeks on both sides of the neck, just below the ears.

The formulation, which was based on hydrophobic-lipophilic balance, is presented in **Table 1.** The two emulsion, oil-in-water (W/O) were based on substances that have been proved to have decent or even no interaction with the skin microbiome, except for the prebiotic ingredients. For control sides, similar formulations not containing active ingredients were produced and placed in a plastic bottle with a dispensing pump. In this article, the term 'Acid group' refers to the group of participants who used the cream containing a combination of Salicylic and Glycolic acids. And the term 'FOS group' refers to the group of participants who used the cream containing FOS as the active ingredient.

Table 1 Ingredients that are used to make the different creams (in percentage).

Name	Cream (FOS)	Cream (Acid)	Control cream
Water	~ 100	~ 100	~ 100
Fructo-oligosaccharides (FOS)	2	-	-
Salicylic acid	-	1	-
Glycolic acid	-	1	-
Mineral oil	2	2	2
Octyl-1 dodecanal	3	3	3
Caprylic capric triglyceride	7	7	7
Glyceryl mono stearate (GMS)	2.8	2.8	2.8
Macrogol 40 glycerol hydroxy stearate (Kolliphor)	1.5	1.5	1.5
Propanediol 1	2	2	2
Phenoxy ethanol	0.4	0.4	0.4
Sodium benzoate	0.2	0.2	0.2
Triethanolamine 99%	Adjust PH ~ 5.5	Adjust PH ~ 5.5	Adjust PH ~ 5.5

Sample collection, DNA isolation and sequencing

In three sampling runs 36 DNA samples were collected for downstream analysis of 16S rRNA regions using MinION sequencing. The samples were taken in week 0 (baseline), one week after application of the cream and two weeks after application of the cream.
The baseline samples were taken using two methods A and B, both swabs were pre-moisturized with a phosphate stabilization buffer (137 mM NaCl, 2.7 mMKCl, 10 mM Na2HPO4, 1.8 mM KH2PO4) + 0.1% tween 20. Method A using a Qiagen Omniswab and B using a cm2 of sterile gauze and long tweezers. The tweezers were rinsed with an
excess of water and sterilized using 70% ethanol before taking each sample. The sterile gauze method was chosen to take the samples for the samples for week 1 and 2 after applying the cream, being the better method in terms of DNA yield.

Two samples were taken per interval (baseline: Friday-Monday week 1: Friday-Monday, week 2: Monday- Friday) per subject to assure enough sample was collected and to counteract some variability and later pooled. Samples were collected using zig-zag strokes on the selected part of the skin (under the ear) and placed inside a 2ml microcentrifuge tube with 500 µl of the phosphate stabilization buffer. Samples were then stored at –20 C until the corresponding second sample was taken. Proteinase k (2µg/ml) was added to both samples and then incubated at 56 C for an hour. After incubation the two samples (of the same subject and same timepoint) were pooled and concentrated down to ±250µl using SpeedVac at 42C for optimal DNA extraction using low DNA yielding skin microbiome samples (Bouevitch et al., 2020). The lysate is used in DNA extraction using the Qiagen PowerSoil Pro kit (Qiagen, Hilden Germany).
The isolated DNA was measured using a Qubit hsDNA. Preparing the samples for 16s sequencing was done using the SQK-RAB204 (RAB_9053_v1_revQ_14Aug2019) for the baseline samples and SQK-16S024 (16S_9086_v1_revW_14Aug2019) (Oxford nanopore technologies, Oxford UK) for the succeeding samples, the number of cycles for the amplification step was changed from 25 to 35.
For the PCR cleanup CleanNA beads were used (CNGS-0050, CleanNA, Waddinxveen, NL). For sequencing the 9.4.1 Flowcell in combination with a MinION sequencer was used (Oxford nanopore technologies, Oxford UK)

Imaging

Photos were taken in a studio with a DSLR with a zoom lens using a shutter speed of 1/160 and an ISO of 1000. The focal length was 120 mm at approximately 2,5 meters distance to the subjects. To achieve optimal conditions for automatic cropping and analyzing the pictures were taken with the following rules: The order of people is always the same, the pictures are taken three times for both sides of the face, the camera 'grid view' is used to align the picture to right part of the neck and
preferably no clothes or jewelry were seen in that part of the grid. All the pictures were taken before sampling DNA from the skin to avoid skin irritation. After collecting picture data, we possessed 5 datasets over 3 weeks including the baseline where each dataset contains 6 photos per person - 36 photos altogether for one data point. Here and after image processing was conducted using Python 3.10 and the OpenCV package (cv2 interface, version 4.5.5.62)

Automatic image preprocessing

The same area on all photos was automatically cropped out with constant rectangle coordinates according to the face side. After that, the red channels of the pictures were extracted, normalized, and converted to the black and white pictures using the mean value of all pixels as the threshold value. The histograms comparing the pixel values distribution on the pictures from the baseline and the coming week datasets were built. In most cases the patterns of the kernel density estimation (KDE) lines on the histograms corresponding to certain subject and the face side stayed the same over time with almost zero lag which was explained as that the automatically cropped pictures captured the same skin area and the illumination conditions were not changed. However, some of the KDE lines repeated the pattern with decent lag which implies the lightning conditions variation or did not match the pattern at all that stands for the shifts in the initially captured skin areas. Therefore, the resulting automatically cropped pictures were not comparable and the idea of manually cropping photos was accepted.

Data preparation for the CCL algorithm implementation

All taken pictures were cropped manually considering the moles as the
control points. After that, the red channels were extracted and adaptive threshold method using Gaussian mean of the pixel intensities was applied in case the illumination conditions were non-uniform across the images over time. As was mentioned above, each time 3 photos per person and face side were taken so the next step was intersecting these 3 pictures and getting one final black and white picture.

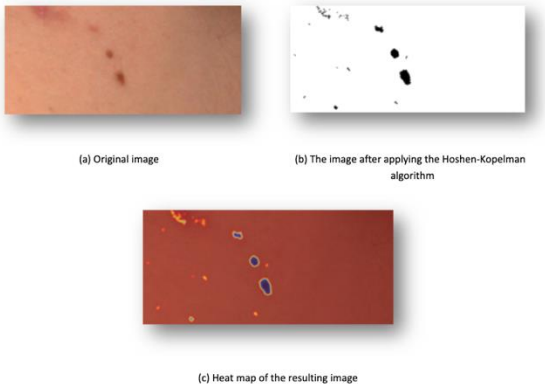

Figure 1 The results of image processing steps.

CCL algorithm implementation

Connected-component labeling, specifically the Hoshen-Kopelman algorithm (*The Hoshen-Kopelman Algorithm*, n.d.) was applied to the resulting images. It labels the clusters of pixels scanning the image and finding the neighbours in the 4-connected neighbourhood which stand for the pixels having a common side but not those sharing only a corner. The pixel clusters were stored in the disjoint-set data structure also called merge-find set to speed up the code performance of merging two equivalence classes - pixel clusters and finding the representative member of the cluster - minimal label value. In the process of labelling the cluster sizes were stored. To eliminate the image noise pixels inside the small clusters were set back to the background color (white). Small clusters are considered clusters with less than 15 pixels. The results of applying the steps described above are shown in **figure 1** (a-b).

Measurements

To find out if the skin irritation appeared over the 3-week period the occupancy percentage of the set pixels was stored. Also, to keep track of the cluster sizes the average amount of pixels per cluster was measured and represented in percentage as a relation to the whole image size. The heat maps of the final pictures were built to illustrate the changes in location and size of clusters over time **figure 1** (c)).

16s rRNA data processing

FastQ files were generated during the MinION sequencing runs (Cock et al., 2010). Several FastQ files are generated for 36 barcodes (from now on called samples). Quality control is carried out using MinIONQC v1.4.2 (Lanfear et al., 2019). It is a recently generated quality control build in R rapidly conducting quality control of MinION reads and generating publication ready figures (R Core Team, 2022). Taxonomic labelling of reads is done by Kraken2 v2.1.2 (Wood et al., 2019). This k-mer based approach provides fast taxonomic
classification of reads, among which 16S rRNA reads. Kraken2 provides support for three 16S databases: Greengenes, RDP and SILVA. However, the National Center of Biotechnology Information (NCBI) hold more recent 16S rRNA bacterial records, with subspecies taxonomic levels. Therefore, we locally downloaded the NCBI 16S bacterial database (downloaded on 03-01-2023) (Sayers et al., 2011). Based on the 16S bacterial database a Kraken2 database was build, allowing taxonomic classification of reads for each sample. After this step, Bracken v2.8 was used to classify more reads to the species taxonomic level (Lu et al., 2017). Bracken computes the abundance of species in DNA sequences based on Bayesian estimation. The use of Bracken required a Bracken database build inside our Kraken2 database directory. Lastly, Bracken uses Kraken2 output files to re-estimate the assigned taxonomic levels. Taxonomic classification dependencies are installed using Conda 22.9.0 (Anaconda, 2016). Since we have worked with large amounts of data, high-intensive computing jobs have been performed on the Sample Linux Utility Resource Management v21.08.8 (SLURM) resource manager (Yoo et al., 2003).

Results

MinionQC generated several informative plots, creating an overview of the quality of the reads. Almost all reads have a length of ~1500 bp, as was expected when sequencing 16S rRNA reads (Supplementary figure 3). Read length during the sequencing run is consistent during the sequencing run of T0 and T1 (Supplementary figure 4). Overall, we observe good quality read length of our samples and therefore no further downstream alterations were necessary. During this study classification on a (sub)species level was priority. Therefore, several databases were tested in order to distinguish the performance between databases. Out of Greengenes, RDP and SILVA, Greengenes performed best using the data of this study. Results comparing the classification results of the aforementioned database with the performance of NCBI are depicted below in **figure 2.** NCBI was able to classify abundantly more different species compared to Greengenes. Where Greengenes did not classify more than 500 different species in a single sample, NCBI only has a few samples with less than 500 distinct species. Although Greengenes had a higher overall species classification percentage of reads (Supplementary figure 2), NCBI seems able to identify a more diverse number of species. Samples having a high percentage classified reads could be misleading. Such samples could contain low amounts of reads. Sample 4 failed during the T0 run and only produced 23 reads. Supplementary figure 1 depicts these results.

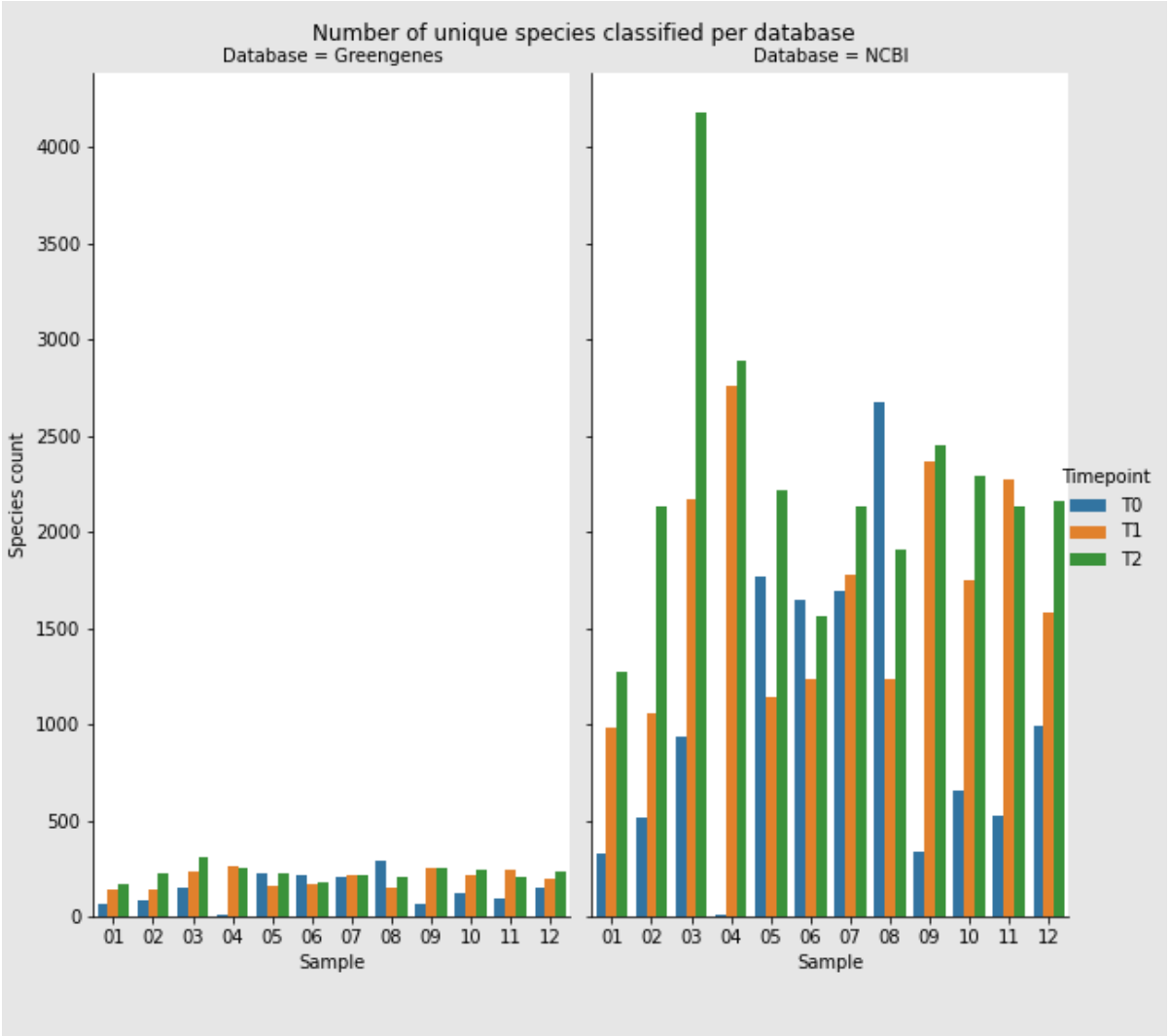

Figure 2 display a Bar chart of the total number of distinct species classified using the Greengenes database (left) and the NCBI database (right) for each sample at each timepoint. The NCBI database was able to classify impressively more distinct species compared to Greengenes database. Sample 3 at T2 was assigned over 4000 distinct species, while Greengenes, for the same sample and timepoint, classified under 400 distinct species.

Microbiome Composition

A total of 1793 different Operational Taxonomical Units (OTU's) ergo bacteria were found in the 16srRNA sequencing results. **Figure 3** shows the bacterial composition with an abundance of over 1% for all participants, over the three weeks of experiments. The majority of the microbiome for all participants is composed of *Cutibacterium acnes* (over 50%), *Staphylococcus pisciferm*entans (around 50%), *Staphylococcus epidermidis* (around 25%), *Staphylococcus hominis*, and *Staphylococcus auricular* (less than 20%). Despite minor changes, they maintained dominance throughout the experiment.

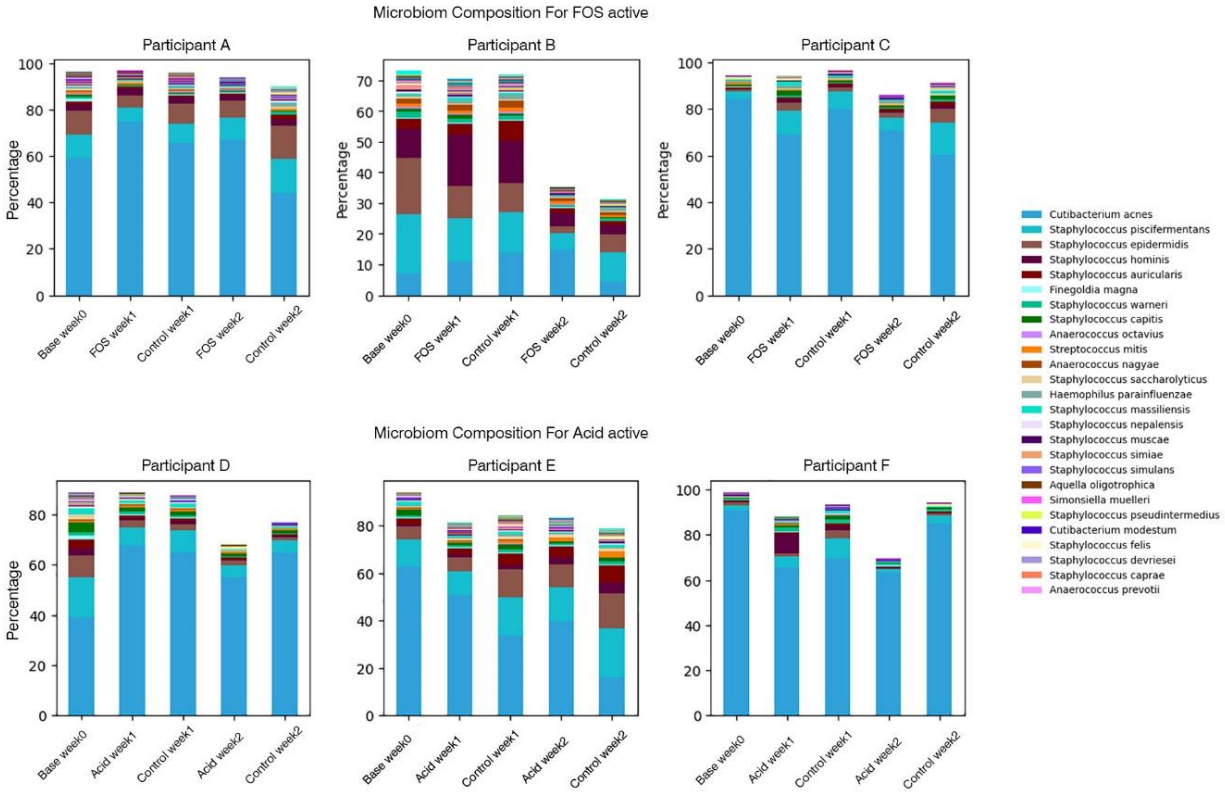

Figure 3 Stacked-bar plot showing the relative abundance of bacteria during the experiment.

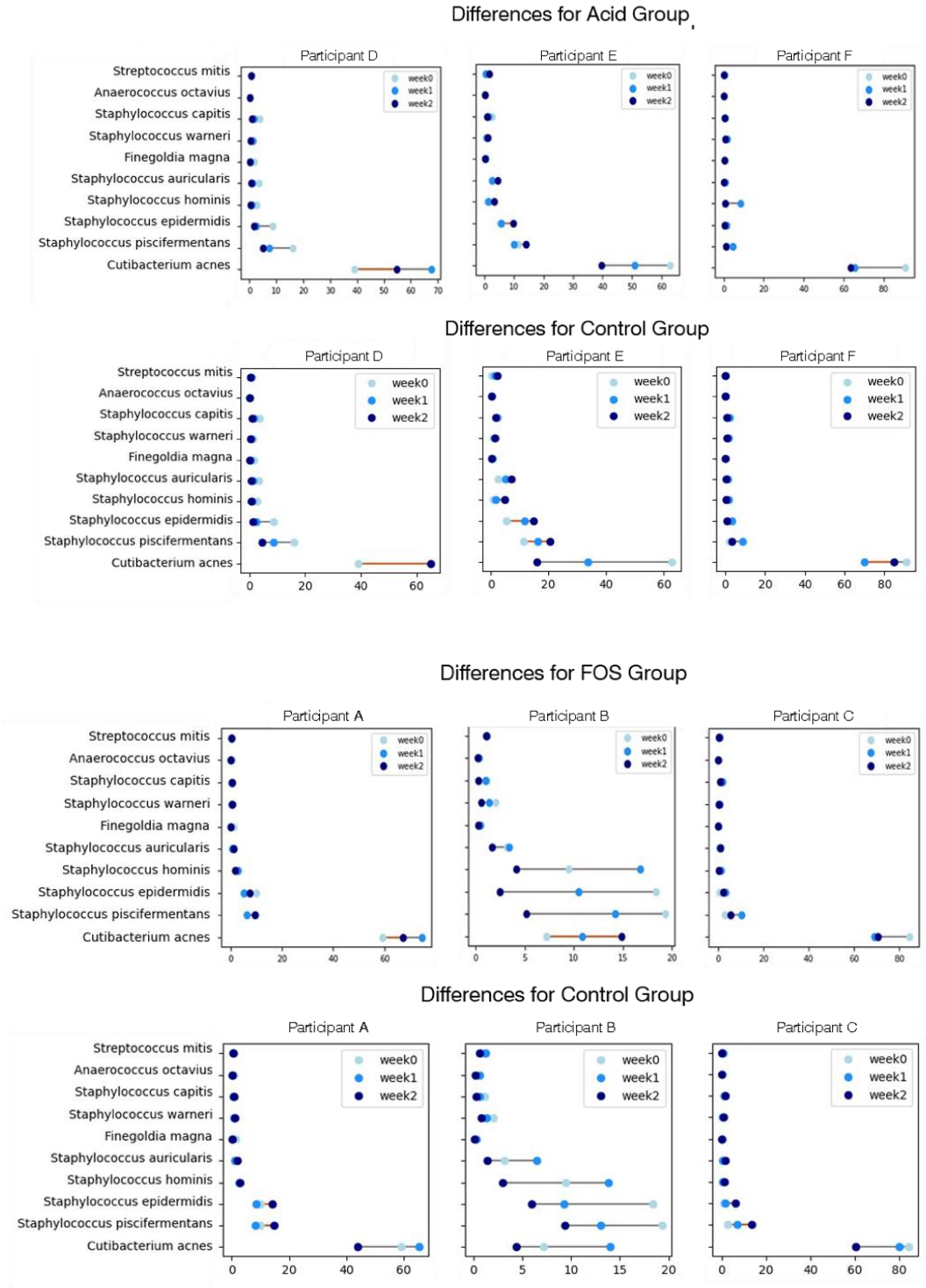

Figure 4 Halter plots showing the changes in abundance over time, relative increases shown in red, decreases shown in grey.

Figure 4 Illustrates the changes in the percentage of microbiomes community, per participant from both groups. Changes of 10 dominant bacteria, particularly *C.acnes* show a negative trend by the end of week 3, for all participants, except participant B, where a 7.5% growth is seen. Though the percentage of *C.acnes* for this participant was lower to begin with.

Microbiome Diversity

Figure 5 shows the Shannon index and the inverse Simpson index plots for participants C and D. A two-sample t-test was performed to compare the active creams to both the control cream and the baseline (Supplementary table 1). No statistical evidence is found to support the idea that applying one of the creams increases the biodiversity of the skin microbiome compared to the baseline and the control(base). The inverse Simpson index was taken instead of the Simpson index for the purpose of displaying the results. As the Simpson index ranges from 0 (infinite diversity) to 1 (no diversity).

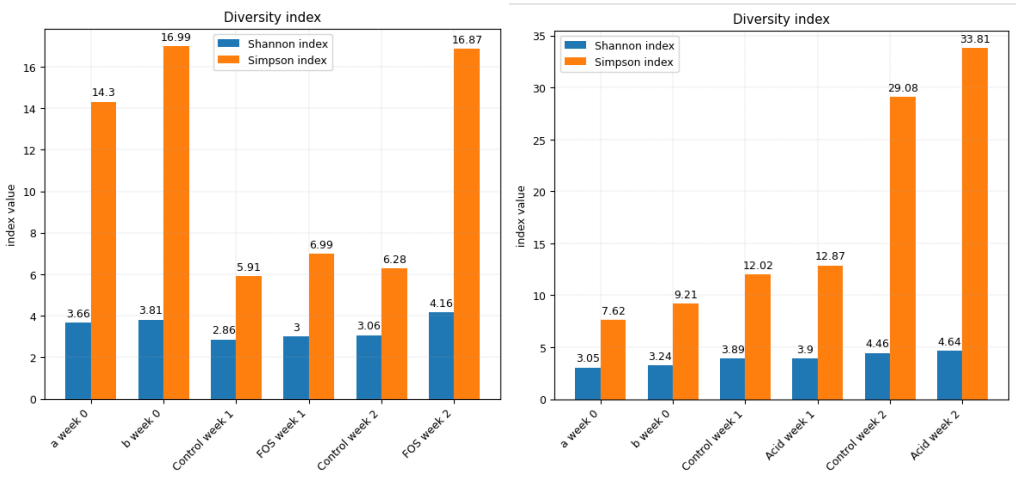

Figure 5 Shannon and Inverse Simpson indexes for participant C (left) and participant D (right).

Image analysis

Figure 6 depicts the results for all participants in the Acid group. The red lines in the plots represent the percentage of red occupancy area of the active side (the side of the neck that was treated with the cream containing the active ingredient). These lines do not show any increase or decrease across all the graphs. This is also true for the percentage of red occupancy area of the control side (blue lines). This indicates that the ingredients in the acid creams (Glycolic and Salicylic acid) did not visibly harm the participants' skin. The base cream also behaved (blue line) similarly, with no discernible impact recorded (**figure 6**).
Furthermore, this is supported by the purple lines, which represent the average area size in percentage of red spots for active cream. This line exhibits neither an increase nor a decline across all the plots. The same results were captured for the black line that represents the red spot's average area size percentage of the base cream. The red spot area size was practically identical between the active cream face side and the based cream face side. This illustrates that the red spots did not enlarge and that the active and base cream had little to no visible effects on red regions that were already present.

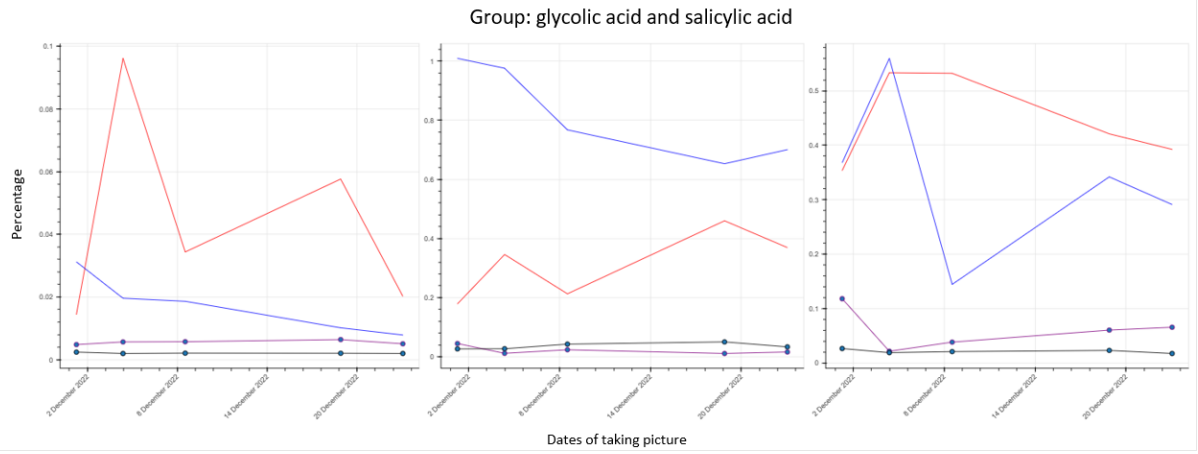

Figure 6 Left to right, displays the results of the Acid cream applied by the three participants nr D, E and F. The Y axis shows percentages, and the X axis shows the dates at which pictures were taken. The blue lines represent the red occupancy (%) for the base cream, the red lines show the red occupancy (%) for the acids cream. The purple and black lines represent the red spot average area size (%) for the base and active cream respectively.

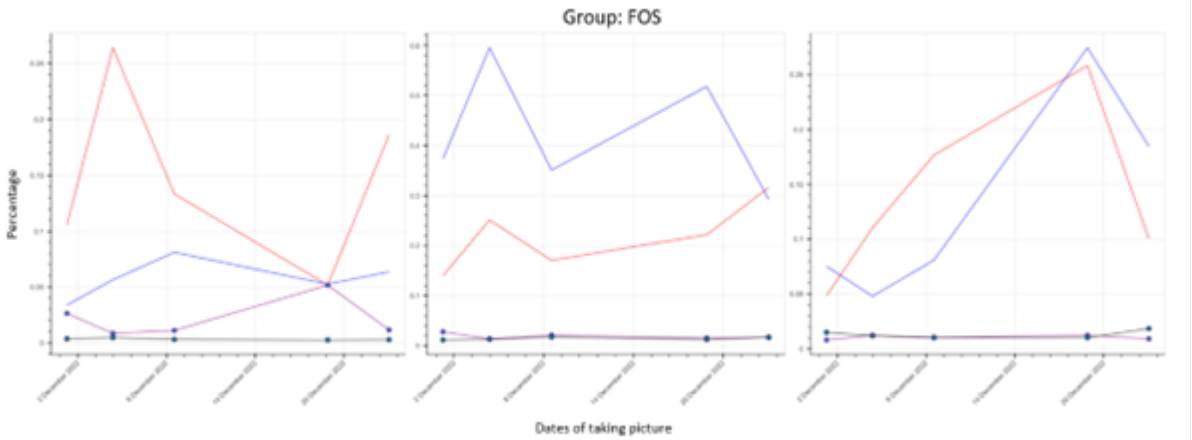

Figure 7 Left to right, the three participants A, B and C applying a cream with FOS. The Y axis showing percentages and the X axis show the dates at which pictures were taken. The blue lines represent the red occupancy (%) for the base cream, the red lines show the red occupancy (%) for the FOS cream. The purple and black lines represent the red spot average area size (%) for the base and active cream respectively.

Figure 7 depicts every member of the group who applied the creams produced from FOS. The red lines in the plots represent the red occupancy area percentage of the active cream like in **figure 6**. As observed in **figure 7** plot 1, the red line is gradually falling but eventually rising again. This may indicate that the cream is affecting the skin of this participant. The subject depicted in **figure 7** plot 2 exhibits a similar, but opposing results, with a gradually increasing red occupancy area percentage of the active cream. The last participant shows a gradual increase in the percentage of red occupancy area for the active cream, followed by a significant decrease at the end. Except for **figure 7** plot 3, where both the red and blue line show a gradual increase and then decline at the end, the gradual increase of red occupancy area percentage of the base cream (blue lines) does not exhibit any significant increase or decrease across all plots.
The average area size percentage for the red spots (black and purple lines) do not consistently rise or decrease across all plots. The slight increase in **figure 7** plot 1 could be attributed to acne or another red spot that was photographed at that precise moment.

Discussion

The selection of preservatives was a sensitive subject for the production of the creams. Since it was not possible to create the product without a preservative, and it was also crucial to ensure that the desired active material is the only effective factor (positive or negative) on the target bacteria (Pinto et al., 2021).

Classification

Creating a map of the human skin microbiome is a key aspect when one studies the effect of prebiotic compounds. During this study we were able to classify a high percentage of bacterial reads, yielded as described in the method section, on a species level. Using the 16S bacterial database of NCBI also enabled classification of subspecies. Kraken2 in combination with Bracken were able to quickly build local databases, followed by rapid classification of the 16S bacterial reads. Subspecies of C. Acnes have been found among all samples during. Recent studies investigated human skin disorders associated with subspecies of C. Acnes (Ahle et al., 2022; Spittaels et al., 2020). Although 16S rRNA sequencing and classification is a proven method for mapping the human skin microbiome, subspecies (gene)analysis having only 16S genes can be a challenge. Therefore, further research gathering genetic information from (sub)species of interest either via cultivation or gathered from the environment is recommended. This allows whole genomes sequencing, enabling precise analysis on gene level. A recent skin microbiome single-cell genomics study was able to, using whole genome sequencing, identify intra-species differences on a genetic level (Ide et al., 2022). Cultivation methods of bacterial strains found on the human skin microbiome must be precise and thoroughly conducted. A recent study showed the possibility to swiftly and accurately identify strain variation of cultivated bacterial strains of common (skin) microbiome species (Fleming et al., 2021).

Microbiome

We did a comparative analysis of changes in the abundance and diversity of skin microbiomes after the application of two different prebiotic creams.
The results of halter graphs can be interpreted in favor of positive impacts of prebiotic creams on the decrease of *C. acnes*. In both groups, halter plots show the decrease in the percentage of *C. acnes*. Undesirable growth of these bacteria (Red lines) was observed only in participant B from the FOS group. However, the sample size of only three participants is too small to observe a significant difference in the

decrease of *C.acnes* between the active creams and the control cream.

In evaluating the diversity of the microbiome, no difference was found between the control, baseline and active cream. A potential factor that could have influenced the calculation of the indexes is the number of reads in the samples. Given the fewer reads may result in some bacteria falling below a threshold in comparison to more prevalent species. A Particular problem in this regard could be that our baseline is amplified with fewer PCR cycles than the subsequent samples. However, this would only make an increase in diversity for the active creams less likely as the diversity indexes in baseline samples could, in theory, only be higher. For future studies it is recommended to keep PCR and sequencing conditions as even as possible for all samples. Another recommendation is the validation of the method using a mock community, to check whether the abundance of some microorganisms is misrepresented in the data.

In conclusion, while this study failed to reject the null hypothesis that prebiotic creams have no significant effect on the relative abundance of *C.acnes*. Additionally, it is important to note that the effects of diet and other daily routines may also play a role in the changes observed in the skin microbiome. To statistically prove or disprove the hypothesis that the microbiome diversity increases after application of a prebiotic cream a larger sample size is required, for in this experiment the number of participants for one cream was only three. Future studies could consider collecting information on participants' dietary habits and daily routines and incorporating these variables into the analysis to gain a more comprehensive understanding of the effects of prebiotic creams on the skin microbiome.
It is also worth considering the type of prebiotic ingredient used in the cream and its effect. It could be interesting to investigate the effect of prebiotic creams in combination with other treatments, such as probiotics or antibiotics, to see if this could enhance the effect on the microbiome.
Claims made by skincare companies regarding the benefits of prebiotic creams may be due to a specific combination of ingredients, not just the presence of prebiotics.

Image analysis

The ability of salicylic acid to reduce acne has led to its widespread use in the cosmetics industry. By lowering swelling and redness and clearing clogged skin pores, salicylic acid helps to treat acne and encourages pimples to shrink. Salicylic acid's effects have been seen and researched in the past. It demonstrated that after 28 days of acid application, the influence of the acid can make pimples on the skin smaller (Dreno B et al., 2017). Salicylic and glycolic together were anticipated to help reduce the size of pimples (Arif, 2015; Lu et al., 2019; Sarkar et al., 2019). Salicylic acid and glycolic acid had little to no impact on the individuals' skin redness occupancy and average red spots area. The variations in each graph in **Figures 6 and 7** are not caused by the cream's active ingredient, as it can be deduced from the fact that the base cream's redness occupancy percentage also fluctuated, indicating that the active component had little to no visible impact on the skin. Additionally, the active cream's red spot average area size percentage and the base cream's red spot average area size percentage were both incredibly low (under 1 percent). proving that the cream's active ingredient had no discernible effect on the existing red spots and had not caused any new ones to appear. For the second cream, this outcome remained consistent. The second cream had the same base cream as the one containing salicylic and glycolic acids, but its active component was fructooligosaccharides, or FOS in short. FOS is one of the first ingredients designed to feed the bacteria that live on the skin. Meaning that undesirable bacteria like *Cutibacterium acnes* will also benefit from the additional nutrients given and not just the healthy bacteria already present on the skin. Red patches and zits are typically brought on by the *Cutibacterium acnes*. However, the FOS in the cream had no discernible impact on the participants' skin, as shown in **figure 7**. The base cream's red occupancy percentage and the active cream's red occupancy percentage were both fluctuating. Showing that FOS was not the cause of the redness captured. With the average spot size, this was still valid. All the plots in **figure 7**'s lines showed low values for both the red spot average area size percentage of the active cream and the red spot average area size percentage of the base cream. The same results were shown in a previous done study by (le Bourgot et al., 2022) According to their findings, FOS promoted and maintained *Staphylococcus epidermidis'* development for up to 24 hours when it was present in concentrations of 0.5 to 5 percent. Contrarily, no FOS prebiotic effect was seen *Cutibacterium acnes*. Showing that FOS specifically boosted the growth of *Staphylococcus epidermidis*, a strain that is thought to be advantageous for maintaining the skin microbiota's homeostasis (le Bourgot et al., 2022). The subjects' skin responded indifferently to both treatments, if at all. The fact that no allergic reaction was observed was viewed as a positive outcome. As previously indicated, the cream's obvious effects were negligible, although this does not preclude eventual effects. The investigation should be carried out over a longer length of time in order to adequately examine this as well as the effects of the creams on microbes.

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