FACULTEIT WETENSCHAPPEN

VAKGROEP BIOCHEMIE EN MICROBIOLOGIE (WE10)
LABO VOOR MICROBIOLOGIE

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DATUM	PAGINA	ONS KENMERK
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Study: Can Synbio Body Cream influence the skin microbiome?

Goal

This study is a proof-of-concept study to demonstrate that the product Synbio Body Cream from the company HeiQ Chrisal, can influence the skin microbiome. The product contains probiotic Bacillus species and inulin as active prebiotic. Upon application of the product, the probiotics should create a protective layer of good microorganisms, whereas the prebiotic sugars should stimulate the growth and diversity of naturally present good microorganisms in the skin microbiome.

The main objective is to show that the probiotic *Bacillus* species from the product can actually survive and be active on the skin; and subsequently that difference in the skin microbiome are witnessed several days after product use.

Test setup

10 test people, equally distributed over gender and of ages between 14 and 62 had to use the Synbio Body Cream on a daily basis for 10 days. Swabs to determine the skin microbiome were taken just before the first product use and 4 days after the last product use. Sampling before/after treatment was done at exactly the same location on the body. Swabs were immediately stored at -20°C before transportation to the lab.

Upon arrival at the lab, all 20 samples were visually inspected to evaluate compliance with sampling and collection protocols before being stored at -80°C (see Table1 for list of samples at arrival).

As communicated, due to worldwide unavailability of extraction kits, a deviation of the proposed extraction protocol was needed to a similar high-quality protocol. Therefore, DNA was extracted from the frozen skin swabs using the RNeasy PowerMicrobiome® Kit (QIAGEN group) according to the manufacturer's instructions, with minor adaptations. A such, a heating step (10min at 90°C) was added after vortexing/bead beating to increase DNA yield, and the DNA removal steps (steps 12 to 16 in the protocol) were excluded.

After DNA extraction the DNA yield was assessed using fluorimetrics analyses (Quantus Fluorometer®) to quantify the double stranded DNA. Individual yield per sample is displayed in Table 1. As none of the obtained yields met the criteria for sequencing, the DNA was further concentrated using vacuum centrifugation and the corresponding concentrations are also displayed in Table 1.

Table 1: Overview of the concentrations and sample names throughout the analyses

Samples at arrival	Sample names for analyses	Concentration after extraction (ng/µl)	Concentration after vacuum centrifuge (ng/µl)
1VOOR	AA	0,246	0,603
1NA	AA	0,126	0,309
2VOOR	BB	0,351	0,860
2NA	BB	0,271	0,664
3VOOR	CC	0,363	0,889
3NA	CC	0,075	0,184
4VOOR	DD	0,207	0,507
4NA	DD	0,134	0,328
5VOOR	EE	0,124	0,304
5NA	EE	0,331	0,811
6VOOR	FF	0,342	0,838
6NA	FF	0,214	0,524
ATV	AT	0,245	0,600
ATN	AT	0,479	1,174
MTV	MT	0,198	0,485
MTN	MT	0,178	0,436
RTV	RT	0,562	1,377
RTN	RT	0,363	0,889
TTV	TT	0,451	1,105
TTN	TT	0,249	0,610

16S rRNA genes amplification and sequencing was outsourced. Sequencing was performed using the Illumina MiSeq platform producing 300bp paired-end reads, targeting the V3-V4 region.

Upon data availability, skin microbiota profiling was performed. The data analyses were conducted in a blinded fashion, as the data analyst had no taxonomic information about the probiotic used. The dual-index data set was preprocessed using the DADA2 pipeline version 1.16. To analyse the microbiota taxonomic composition, taxonomy assignment of sequences was performed next, using the Silva species assignment database version 132, to generate phylum to genus level composition matrices as well as species identification where possible.

Before pair-wise analyses of the samples, quality inspections were performed using Phyloseq in R (data not shown). As the quality of the data was good and no sample processing bias was observed, the pair-wise analyses to assess the impact of probiotic use on the skin was assessed. Two-tailed tests were performed on the relative abundances to reveal compositional changes upon probiotic use. In this pilot study the cut-off for significance was set at 0.1 and no correction for multiple testing was performed in order to allow the detection of changes in this cohort. Only ASV's that were present in at least 20% of the samples were considered.

Ordination of the data using nonmetric multidimensional scaling based on Bray-Curtis distances (Figure 1) shows an overall high similarity of the samples per individual as expected. According to the pre-set analysis parameters, significant changes were observed in 20 ASV's after probiotics application. In total three ASV's were increased after probiotic use (p-values: 0.036, 0.049 and 0.055) and they were all taxonomically assigned to the *Bacillus* genus. This increase in *Bacillus* spp. was at the dispense of 17 ASV's which were all assigned to common skin bacteria. The most pronounced impact was seen on *Anaerococcus*, *Cutibacterium granulosum*, *Rothia* and *Staphylococcus* (respective p-values: 0.041, 0.022, 0.046, 0.050).

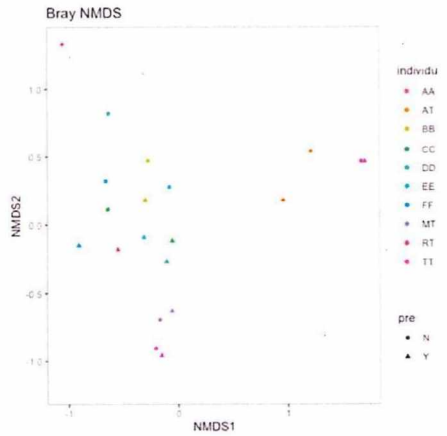

Figure 1: Ordination of the data using Bray-Curtis distance

Conclusion

From this proof-of-principle study it can be concluded that the Synbio Body Cream enriches *Bacillus* species on the skin. As this enrichment was detectable 4 days after cessation of the application of the probiotic cream, this suggests that the probiotic cream impacts on the existing skin microbial community. Furthermore, ordination of the data suggests unidirectional changes in the skin microbiome after using the product for 10 days, hinting towards specific modulations. More detailed studies are required to gain more insight in the details of the skin microbiome modulation (ex. differences between sexes) and to determine adequate sample sizes for follow-up studies.

Met vriendelijke groeten

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