

Evaluation of the effects of age, gender and dexpanthenol containing skincare on the facial and body skin microbiome

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Supplementary Information

S.1 Test product ingredients

Ingredient lists for the test products are given below;

DFCD – Dexpanthenol repair complex (DRC) Face cream day

Aqua, Caprylic/Capric Triglyceride, Glycerin, 1,2-Hexanediol, Butyrospermum Parkii Butter, Dexpanthenol, Polyglyceryl-6 Distearate, Cetearyl Alcohol, Isopropyl Isostearate, Niacinamide, Squalane, Jojoba Esters, Tocopheryl Acetate, Glyceryl Stearate Citrate, Behenyl Alcohol, Acacia Senegal Gum, Polyglyceryl-3 Beeswax, Cetyl Alcohol, Xanthan Gum, Argania Spinosa Kernel Oil, Citric Acid.

DFCN – DRC Face cream night

Aqua, Caprylic/Capric Triglyceride, Glycerin, 1,2-Hexanediol, Butyrospermum Parkii Butter, Cetearyl Alcohol, Dexpanthenol, Squalane, Polyglyceryl-6 Distearate, Isopropyl Isostearate, Isosorbide Dicaprylate, Niacinamide, Jojoba Esters, Behenyl Alcohol, Glyceryl Stearate Citrate, Tocopheryl Acetate, Acacia Senegal Gum, Polyglyceryl-3 Beeswax, Cetyl Alcohol, Xanthan Gum, Argania Spinosa Kernel Oil, Citric Acid.

DFC-SPF – DRC Face cream (SPF 25)

Aqua, Homosalate, Dibutyl Adipate, Butyl Methoxydibenzoylmethane, Ethylhexyl Salicylate, Glycerin, Caprylic/Capric Triglyceride, Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine, Silica, 1,2-Hexanediol, Dexpanthenol, Ethylhexyl Triazone, Polyglyceryl-6 Distearate, Niacinamide, Behenyl Alcohol, Cetearyl Alcohol, Isopropyl Isostearate, Jojoba Esters, Butyrospermum Parkii butter, Potassium Cetyl Phosphate, Tocopheryl Acetate, Acacia Senegal Gum, Polyglyceryl-3 Beeswax, Cetyl Alcohol, Xanthan Gum, Argania Spinosa Kernel Oil, Squalane, Tetrasodium Glutamate Diacetate, Citric acid.

DFW – Dexpanthenol Face wash

Aqua, Lauryl Glucoside, Sodium Cocoamphoacetate, Glycerin, Dexpanthenol, Disodium Cocoyl Glutamate, 1,2-Hexanediol, Caprylyl/Capryl Glucoside, Guar Hydroxypropyltrimonium Chloride, Citric Acid, Sodium Chloride, Niacinamide, Xanthan Gum, Argania Spinosa Kernel Oil, Lysine.

DBL – DRC Body lotion(low lipid level)

Aqua, Caprylic/Capric Triglyceride, Glycerin, 1,2-Hexanediol, Butyrospermum Parkii Butter, Dexpanthenol, Isopropyl Isostearate, Niacinamide, Polyglyceryl-6 Distearate, Cetearyl Alcohol, Squalane, Jojoba Esters, Tocopheryl Acetate, Glyceryl Stearate Citrate, Behenyl Alcohol, Polyglyceryl-3 Beeswax, Cetyl Alcohol, Argania Spinosa Kernel Oil, Xanthan Gum, Acrylates/C10-30 Alkyl Acrylate Crosspolymer, Citric Acid.

DBH – DRC Body balm (high lipid level)

Aqua, Butyrospermum Parkii Butter, Glycerin, Caprylic/Capric Triglyceride, Isopropyl Isostearate, Niacinamide, 1,2-Hexanediol, Cera Alba, Dexpanthenol, Squalane, Polyglyceryl-6 Distearate, Cetearyl Alcohol, Isosorbide Dicaprylate, Jojoba Esters, Tocopheryl Acetate, Glyceryl Stearate Citrate, Behenyl Alcohol, Polyglyceryl-3 Beeswax, Cetyl Alcohol, Argania Spinosa Kernel Oil, Xanthan Gum, Acrylates/C10-30 Alkyl Acrylate Crosspolymer, Citric Acid.

DHC – DRC Hand cream

Aqua, Caprylic/Capric Triglyceride, Glycerin, Butyrospermum Parkii Butter, Isopropyl Isostearate, 1,2 Hexanediol, Dexpanthenol, Squalane, Polyglyceryl-6 Distearate, Cetearyl alcohol, Niacinamide, Silica, Argania Spinosa Kernel Oil, Jojoba Esters, Glyceryl stearate citrate, Behenyl Alcohol, Tocopheryl acetate, Polyglycerol-3 Beeswax, Cetyl Alcohol, Xanthan Gum, Acrylates/C10-30 Alkyl Acrylate Crosspolymer.

DBW – Dexpanthenol Body wash

Aqua, Capryl/Capramidopropyl Betaine, Lauryl Glucoside, Disodium Cocoyl Glutamate, Glycerin, Dexpanthenol, 1,2-Hexanediol, Sodium Chloride, Guar Hydroxypropyltrimonium Chloride, Citric Acid, Niacinamide, Xanthan Gum, Argania Spinosa Kernel Oil, Lysine.

S.2 Microbiome assessment

PCR was done with the forward primer V3V4_skin_F and reverse primer V3V4_skin_R with Illumina adapters attached.

Illumina adapter and V3V4_skin_F:

5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGAGGCAGCAG-3'

Illumina adapter and V3V4_skin_R:

5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACCVGGGTATCTAAKCC-3'

These are universal bacterial 16S rDNA primers, which target the V3-V4 region. The following PCR program was used: 98 °C for 30 sec, 32x (98° C for 10 s, 55 °C for 20 s, 72 °C for 20 s), 72 °C for 5 min. Amplification was verified by running the products on an agarose gel. Indices were added in a subsequent PCR using an Illumina Nextera kit with the following PCR program: 98 °C for 30 sec, 8x (98° C for 10 s, 55 °C for 20 s, 72 °C for 20 s), 72 °C for 5 min. Attachment of indices was verified by running the products on an agarose gel. Products from the nested PCR were pooled based on band intensity and the resulting library cleaned with magnetic beads. The DNA concentration of pooled libraries was measured fluorometrically. Sequencing was done on an Illumina MiSeq desktop sequencer using the MiSeq Reagent Kit V3 (Illumina) for 2x 300 bp paired-end sequencing.

A customized pipeline based on dada2 was used for bioinformatics processing of the sequence data into an ASV (amplicon sequence variant) abundance table [Callahan et al. 2016, PMID: 27214047]. In a first step, primer sequences were removed from raw reads using cutadapt. Reads without primer match or with ambiguous bases (e.g. Ns), as well as reads shorter or longer than expected from the number of sequencing cycles and the length of the primers were filtered out. In an additional filtering and trimming step (dada2::filterAndTrim command), reads were trimmed at the 3' end based on sample-specific quality scores. Trimmed reads that based on the quality scores of the nucleotides were expected to contain more than one error were removed. The remaining reads were dereplicated into unique sequences and then denoised separately for forward and reverse reads for each sample. In this denoising step, a less abundant sequence can be assigned to a closely related more abundant sequence based on comparison to a data-based error matrix. In that case, the low abundance sequence is considered a sequencing error of the more abundant sequence. Denoised forward and reverse reads were merged thereby discarding read pairs without sufficient overlap or with any mismatch in the overlap region. Finally, suspected chimeras (also called bimeras) were removed from the generated abundance table by internal abundance and sequence comparisons.

The taxonomic assignment of the detected ASVs was done in 2 steps. First, the ASV sequences were compared to full-length 16S sequences in an internal reference database (CM_16S_27Fto1492R_v2.0.0) using a naïve Bayesian classifier. The reference database was generated using in-silico amplicon extraction from the GTDB database (release: 07-RS207, <https://gtdb.ecogenomic.org/>), the rrnDB (version 5.8, <https://rrnodb.umms.med.umich.edu/>), and the UHGG database (v2.0, <https://www.ebi.ac.uk/metagenomics/genomecatalogues/human-gut-v2-0>) and subsequent curation. In the second annotation step, the taxonomic assignments were improved using precise sequence identity percentages between the found ASVs and reference amplicons in an internal V3V4 amplicon database (CM_16S_341Fto785R_v2.0.0.rds).

To test whether alpha diversity measures or taxa abundances were significantly different between groups, the paired Wilcoxon signed rank test was used. When testing beta diversity shifts between groups, permutational multivariate analysis of variance (PERMANOVA) tests were performed using the adonis2 function from the vegan R package with 1000 permutations and by = "margin", thus assessing the marginal effects of the terms (i.e. each marginal term analyzed in a model with all other variables).

When performing statistical testing on multiple hypotheses, we used the Benjamini–Hochberg (BH) method to control the false discovery rate (FDR) at a level of 10%. Thus, of all the “statistically significant” associations we report in this context, we expect 10% of these to be false associations (arising due to chance). FDR control was applied to each tested contrast individually.

Boxplots display the inter-quartile range (IQR; upper and lower box edges), the median (horizontal line inside box), and the extreme of the data observations within $1.5 \times \text{IQR}$ (whiskers). Observations beyond the whisker range are indicated with individual points.