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Topical Application of Synbiotic Bacillus Preparations Positively Affects Skin (Micro) Biology

Ze'evi Ma'or[1], Robin Temmerman[2], Xinmin Zhang[3]

[1]The Laboratory for Biochemistry and Biotechnology of The Skin, The Dead Sea & Arava Science Center, Masada, Israel
[2]HeiQ Chrisal Synbiotics R&D Centre, Lommel, Belgium
[3]BioInfoRx Inc., Madison, USA
Email: zeevimaor1983@gmail.com, robin.temmerman@heiq.com, xinmin@bioinforx.com

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Abstract

Alteration in skin microbiome profile is involved in many skin conditions, therefore, microbiome modulation is a reasonable target for skin health. Probiotic skincare was suggested, and leave-on synbiotic skincare preparation, blending bacillus spores, as probiotic active ingredient, and inulin sugar, as prebiotic booster, is evaluated, using microarray, 16S gene sequencing, and clinical skin analyses. Topical application of leave-on synbiotic skincare on skin model activates a profound effect on skin biology, expressed in the transcriptome level, with higher than 10% affected genes. The significance of the synbiotic preparation on skin biology was assured, indicating the involvement of major biological pathways. Blending probiotic with Polygonum aviculare plant extract, had triggered a distinct gene expression alteration, demonstrating the sensitivity of skin responses to different active substances. A synbiotic skincare application, had successfully introduced live and active *Bacillus* probiotics on human skin, detectable days after application was finalized. Following synbiotic application, the microbial content of several microorganisms, related to skin pathologies, was reduced, while the content of bacillus species, representing a healthier microbiome status, was increased, suggesting that frequent application may lead to overall healthier skin microbiome. Acne vulgaris involves unbalanced skin microbiome, with expansion of Cutibacterium acnes. The efficacy of a synbiotic skin cream was assessed to improve acne symptoms, including IGA dermatologist acne severity score, the number of counted acne lesions, measured skin oiliness and skin redness, visual appearance of skin-pores, skin smoothness and softness, and Acne Quality of Life Index.

Keywords

Probiotic Cosmetics, Skin Transcriptome, Bacillus Ferment, Polygonum Extract, Acne Vulgaris, Prebiotic, Synbiotic Skin Cream

1. Introduction

The human microbiome plays an important role in our overall health, and connections between different parts of the microbiome are being discovered [1]. Microbiome, also known as "the hidden organ", refers to the collection of genomes from a vast number of bacteria, yeasts, molds, and virus in the body environment, including the community of the microbiota, as well as the microbial structural elements and metabolites. Alterations in microbiome can affect different aspects of the host, while its dysbiosis is related to a wide spectrum of diseases [2]. The growing medical understanding in host-microbiota relationship has lately led to the development of microbiota-based therapy such as fecal microbiota transplant (FMT) and probiotic bacteria modulation [3].

More specifically, skin coexistence with microbiota is well established [4]. Alteration in skin microbiome profile is involved in atopic dermatitis, acne vulgaris, rosacea, seborrheic dermatitis, pityriasis versicolor, psoriasis, lupus erythematosus, diabetic foot ulcer, actinic keratosis, and cutaneous conditions in general [5]. Therefore, skin microbiome preservation or modulation as a target to maintain skin health is a plausible approach. Various microbiome related protective and preventive strategies had been proposed, distinguished by their degree of intervention, from non-destructive "microbiome friendly", to moderate active prebiotic, and up to highly active topical application of probiotic live bacteria [6] [7] [8].

Skin microbiome modulation research and commercial trends are supported by its emerging growth in public awareness. In 2021, Google data searches for "probiotic" and "prebiotic" skin care have increased by 70% and 200% respectively [9], and Vogue Magazine lists the probiotic trend among "The 10 skincare trends that will dominate 2023" [10].

Synbiotic skincare was recently introduced by Temmerman and Meersman [11]. Using synbiotic cleaning products to positively influence microbiomes at hospitals was proven [12]. In this work we present results that indicate that leave-on synbiotic skincare preparations, based on blending bacillus spores with inulin, may positively influence human skin microbiome, and related skin conditions. The extract of Polygonum plant is known for its wound healing capabilities [13] [14]. Blending Polygonum together with Synbio could exhibit the potential of using bacteria spores, formulated with other active ingredients, evaluating the different resulted effect on skin. In this report, data include microarray results on 3D skin models, as well as clinical tests on human skin, evaluating the potential effect of a synbiotic blend, and its formulated preparations, to im-

prove skin microbiome and skin conditions. The biological and microbiological effects, following the topical application of live synbiotic preparations is presented and its possible practice in specific skin conditions is discussed.

2. Methods

2.1. Tested Materials

Several commercial and non-commercial preparations, were used including:

1) Synbiotic concentrate—A commercial eco labeled blend (Synbio® Cosmetic Concentrate, HeiQ Chrisal, Lommel, Belgium) of Bacillus spores belonging to four species, B. subtilis, B. licheniformis, B. megaterium and B. amyloliquefaciens, and inulin as prebiotic sugar. This concentrate is dissolved in demineralized water up to a Bacillus concentration of 10E9 CFU/ml and containing an inulin concentration of 1%. The synbiotic concentrate is not an end-product and needs to be processed into final product formulations such as cosmetic creams or lotions.

2) Polygonum aviculare plant extract: a non-commercial extract of Polygonum, grown by Moraz in the Galilee and Jezreel Valley, in the north part of Israel and processed in a unique manufacturing protocol for skin care and dermatological treatment. Plants are cultivated by Moraz in its fields, collected and extracted as described: set a boiler with water, when the temperature is right, a fresh volume of plants is inserted in special baskets and soaked in the boiling water, for couple of hours and decocted for extraction through a special sieve, finalized with a physical filtration.

3) Polygonum aviculare plant extract—Synbiotic concentrate on-commercial blend, specially prepared for this work.

4) Polygonum aviculare plant extract—Synbioic concentrate non-commercial 2:1 blend, specially prepared for this work.

5) Synbio® skin cream—a commercial synbiotic skin cream (HeiQ Chrisal, Lommel, Belgium), an oil in water emulsion, containing 1% of Synbiotic concentrate (*i.e.*, Bacillus ferment, and Inulin), and the following INCI list of ingredients: Water, Glycerol, Caprylic/capric triglyceride, Sodium polyacrylate, Ethyl hexyl stearate, Trideceth-6, Phenoxyethanol, galacto oligosaccharide, Ethyl hexyl glycerol, and Fragrance

2.2. Micro Array Studies

2.2.1. Reconstructed Skin Model

Reconstructed skin, consist of both epidermis and dermis tissues (MatTek EFT-400 full thickness skin tissue) was used as described in manufacturer manual and slightly modified as described in Ma'or *et al.* [15]. Skin pieces were placed into a 6-well plate containing 2.5 ml of assay medium and incubated overnight at 37˚C and 5% CO_2. Then the assay medium was replaced with 5 ml of fresh medium and the tissues were topically treated with tested materials for 24hours. At the end of the incubation period, the surfaces of the tissues were rinsed with PBS

to remove the test materials, and the tissues were homogenized for RNA isolation.

2.2.2. Skin RNA Isolation Protocol

Total RNA was extracted from reconstructed skin, exposed to tested material for 24 hours, using RNAqueous kit (Ambion), followed by mRNA Amplification protocol (Ambion, MessageAmp aRNA kit). Total RNA was reverse-transcribed to cDNA, using a T7 oligo (dT) primer. Second-strand cDNA was synthetized, and *in-vitro* transcribed to anneal RNA (aRNA) and processed following Cox and Singer [16] with slight modifications, as described in Ma'or *et al.* [15].

2.2.3. Detection of Gene Expression/Microarray protocol:

To analyze the topical application effect of tested materials on skin transcriptome, the expression level of 19,254 human genes was identified, using DNA Microarray Chip (human GE 4X44K) from Agilent, following the manufacturer instructions, slightly modified as described in Ma'or *et al.*, [15]. Skin cDNA was obtained via reverse transcription and labeled with Cy3 and Cy5 fluorescent dyes (PerkinElmer, ASAPRNA Labeling kit) and purified by Millipore Micron YM-30 filter column and TE buffer (Invitrogen). After purification, the fluorescent probes were hybridized to the Agilent DNA Microarray Chip for 17 h at 65˚C. Followed by a laser excitation of dye-specific Hz., the microarrays signal was scanned (Axon GenePix, 4100A Scanner) with scanning resolution set to 5 micrometer and analyzed with GenePix Pro 7 Microarray Acquisition and Analysis Software.

2.2.4. Interpretation of Microarray Results

The raw data from each array was processed following Värem *et al.* [17] and Subramanian *et al.* [18] to correct background and normalize within array using the limma R package. From each array the log fold change (LogFC) of treated vs control was calculated for each gene from limma, then an adjusted logFC value is computed by dividing the logFC with relative expression (average expression of the gene versus median expression from the array). Two-fold change is used as cutoff for the adjusted logFC to select up- and down-regulated genes. Gene Set Enrichment Analysis (GSEA) software was used for interpreting the achieved microarray gene expression results, and the significant biological pathways were elucidated. All affected genes were analyzed according to a functional categorization, related to 50 pathways, manually selected by an expert for their relevance to skin's biology from KEGG and Wiki-pathways lists. Enrichment of gene sets was considered as statistically significant if the false discovery rate (FDR) was <0.05.

2.3. Synbio® Skin Cream Effect on Skin Microbiome

2.3.1. Clinical Test Protocol, Skin Sampling and Lab Process Methodology for Microbial DNA Extraction

Synbio® Skin Cream [supplied by HeiQ-Chrisal] was applied daily for 10 con-

secutive days by 10 healthy volunteers, aged between 18 and 62, equally distributed over genders. Swabs to determine the skin microbiome were taken before first application and 4 days after the last product application. Sampling before/after treatment was done exactly at the same location on the body. Swabs were immediately stored at minus 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. 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 heating step (lOmin at 90˚C) was added after vortex/bead beating to increase DNA yield, and the DNA removal steps (steps 12 to 16 in the manufacturer's protocol) were excluded. After DNA extraction the DNA yield was assessed using fluorometric analyses (Quantus Fluorometer®] to quantify the double' stranded DNA. 16S rRNA genes amplification and sequencing was performed, using the Illumina MiSeq platform, producing 300bp paired-end reads, targeting the V3-V4 region.

2.3.2. Microbiological Clinical Test Result Data Analysis

Upon data availability, skin microbiota profiling was performed. The dual-index data set was preprocessed using the DADA2 pipeline version 1.16. To analyze 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. 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. Amplicon Sequence Variants [ASV] methods were used due to their capability to the fine distinction between sequences, resolving sequence differences by as little as a single nucleotide change, thus avoiding similarity-based operational clustering units. Only Amplicon Sequence Variants (ASV's) that were present in at least 20% of the samples were considered. Ordination of the data was performed, using nonmetric multidimensional scaling, based on Bray-Curtis distances.

2.4. Synbio® Skin Cream Clinical Test Protocols on Acne and Other Skin Conditions

Synbio® Skin Cream was daily applied at home on facial skin by 31 women volunteers, aged between 15 and 44 years old (mean age: 23 years old), suffering from Acne vulgaris prone skin, *i.e.* presenting oily skin on the face with a minimum sebumetry value of 120 µg/cm^2 on frontal area and presenting dilated

pores, lack of smoothness and softness in the skin on face and with a mild to moderate IGA Score (Grade 2 - 3) proven by a dermatologist. The compliance was monitored through the participants' report on a daily log. Clinical assessments were performed before usage, after one, two and four weeks of application. The different parameters of skin status were assessed by professional employees of an independent skin clinic, using the following methodologies:

- Skin oiliness reduction through instrumental measurements with Sebumeter® SM 815 device.
- Objective assessment of inflammatory and non-inflammatory Acne vulgaris by a professional trained employee.
- Clinical efficacy assessments according to IGA Score by a dermatologist.
- Image analysis of RBX (redness from acne lesions) and porphyrins, of facial images captured with Visia® CR device.
- Acne Quality of Life index questionnaire (Acne-QoL), answered by each participant at the end of clinical study.

3. Results

This paper presents the result of applying synbiotic preparations on human skin and on in-vitro skin models, aiming to determine the effect on the skin transcriptome, skin microbiome, and skin conditions. Several microbiological, clinical, and genomic technologies were applied during the studies.

3.1. Transcriptome Study

To evaluate the transcriptome response to a topical application of Synbio, we had used microarray, enabling to detect the expression-level of most of the human genes, while bioinformatic tools were used to clarify the involved biological pathways and affected functions, as described in Methods.

Effect following skin exposure to bacillus spores' preparation—micro array results.

Following skin exposure to Synbio tested preparation, described in "Tested Materials" in Methods, the differentially gene expression was detected, using high throughput microarray (Agilent DNA Microarray Chip), as described in "Detection of Gene Expression/Microarray protocol" in Methods. From 19,254 total evaluated number of human genes, 2231 genes were responding to Synbio skin exposure, 1598 genes were considered as up regulated and 633 genes as down regulated, taking two folds change from the adjusted Fold Change values as a cutoff. The MA Plot and Box plot are described in **Figure 1(a)** and **Figure 1(b)** below.

3.1.1. Pathway and Function Analysis of Microarray Results

A functional analysis of the detected up and down regulated genes in microarray, was performed as described in "microarray results interpretation" in Methods. Two methods were used to elucidate the involved biological pathways: Gene Set Enrichment Analysis (GSEA) and a skin functional categorization,

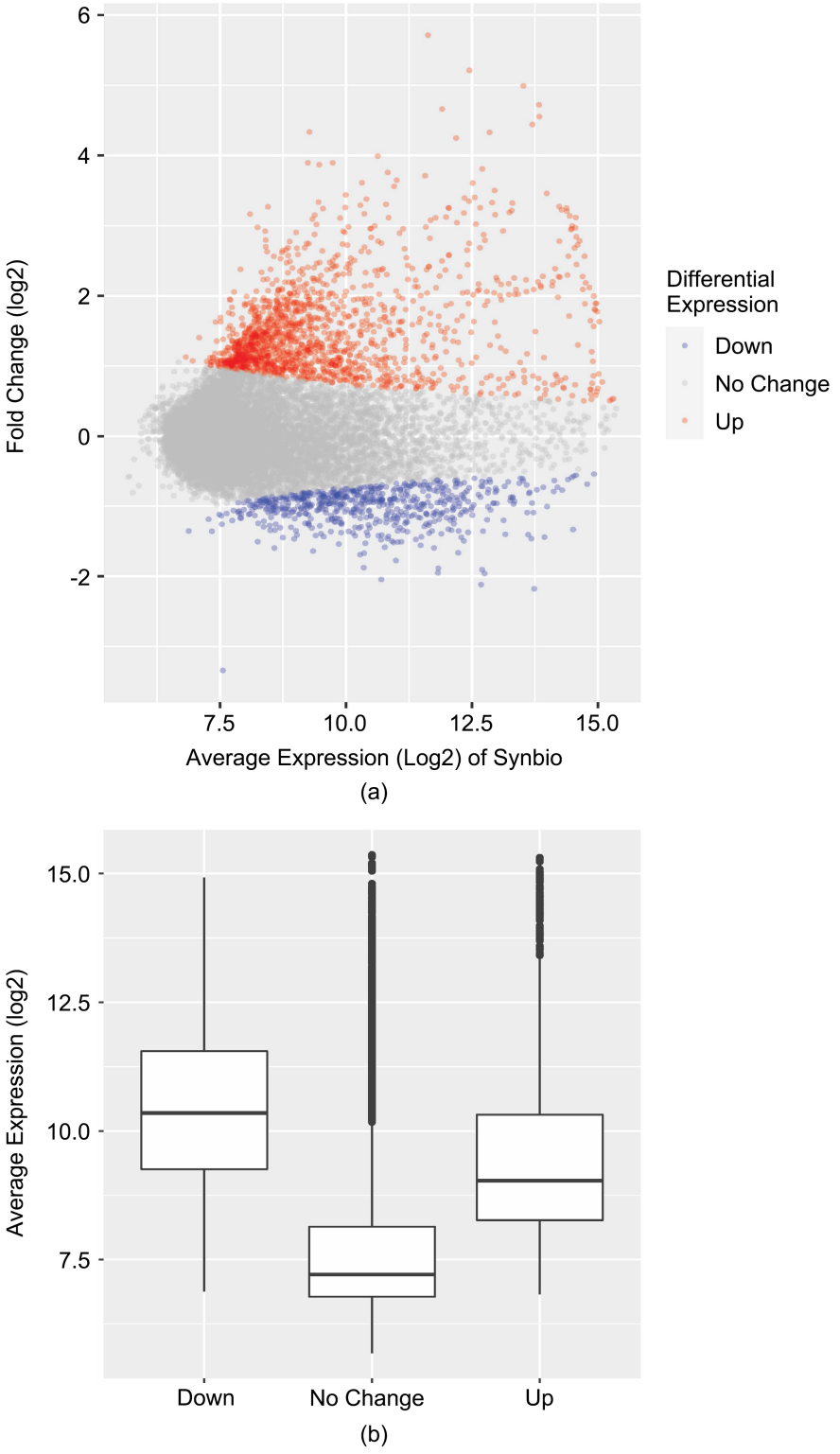

Figure 1. The epigenetic effect following skin exposure to Synbio Bacillus spores' tested preparation. (a) MA plot presents the fold change versus average expression, visualizing the genes that display large-magnitude changes and their expression levels. The up- and down-regulated genes are highlighted. (b) A box plot presents the distribution of expression levels for genes that are up, down, or unchanged, following skin exposure to tested preparation.

related to 50 pathways, selected from KEGG and Wiki-pathways lists, for their relevance to skin's biology. Table 1(a), Table 1(b) and Table 2 below summarize the main involved biological pathways and functional analysis.

3.1.2. GSEA Analysis of Microarray Results

The top functional categories of down (negative) and up (positive) regulated genes from GSEA analysis are presented in Table 1(a) and Table 1(b) respectively.

Table 1. (a) Negative enrichment; (b) positive enrichment.

(a)

Category	Number of Genes in Core Enrichment
HAMAI APOPTOSIS VIA TRAIL UP	286
SEIDEN ONCOGENESIS BY MET	41
WP GLUCOCORTICOID RECEPTOR PATHWAY	25
THAKAR PBMC INACTIVATED INFLUENZA AGE 21 30YO NONRESPONDER 28DY UP	84
SESTO RESPONSE TO UV C0	52
SHEN SMARCA2 TARGETS UP	196
BONOME OVARIAN CANCER POOR SURVIVAL UP	15
TIAN TNF SIGNALING VIA NFKB	9
PATEL SKIN OF BODY ZOSTAVAX AGE 70 93YO VZV CHALLENGE 6HR UP	97

1a: GSEA analysis results, elucidating the top 9 functional categories of down (negative) regulated genes.

(b)

Category	Number of Genes in Core Enrichment
GOMF OLFACTORY RECEPTOR ACTIVITY	128
KEGG OLFACTORY TRANSDUCTION	123
GOBP SENSORY PERCEPTION OF SMELL	132
GOBP DETECTION OF STIMULUS INVOLVED IN SENSORY PERCEPTION	135
GOBP SENSORY PERCEPTION OF CHEMICAL STIMULUS	152
GOCC KERATIN FILAMENT	27
GOMF G PROTEIN COUPLED AMINE RECEPTOR ACTIVITY	19
DESCARTES FETAL INTESTINE VASCULAR ENDOTHELIAL CELLS	12
REACTOME OLFACTORY SIGNALING PATHWAY	15
GOMF ODORANT BINDING	45

1b: GSEA analysis results, elucidating the top 10 functional categories of up (positive) regulated genes.

3.1.3. Functional Categorization

All affected genes were analyzed according to a skin functional categorization, related to 50 skin pathways, selected for their relevance to skin's biology from KEGG and Wiki-pathways lists. Table 2 below summarizes the involved biological pathways, according to their functional categorization.

3.1.4. Transcriptome Effect Following Skin Exposure to a Preparation, Blending Probiotic Bacillus Spores, Prebiotic Inulin and Polygonum Plant Extract—Microarray Results

In parallel research, a Synbiotic preparation, containing a mixture of spores of four specific Bacillus spp. (B. subtilis, B. licheniformis, B. megaterium and B. amyloliquefaciens) with a prebiotic inulin sugar, was mixed with Polygonum aviculare plant extract as described in "tested materials" in Methods. Following skin's topical exposure to the tested blend, a pattern of transcriptome was found, presenting the ability to stimulate beneficial skin effects, that are largely different from the achieved results of the application of the single ingredients, Synbio or Polygonum, alone (data not shown). The application of the new blend could lead into the involvement of molecular pathways, that impact skin ability to neutralize free radicals, to reduce UV damage, to control inflammation, to balance keratinocyte differentiation, and to affect skin capacity of self-renewal. Based on the analysis, using advanced data-mining tools, the involvement of specific functional pathways was suggested, that may affect the regulation of cell cycle and pluripotency and differentiation of keratinocytes, as well as regulate skin biosynthesis and metabolism of some important bio components, such as retinol, collagen, and radical scavenging mediators. Founded on this microarray research, a patent was applied by a commercial skincare company (Moraz US patent pending 63/376,477).

Table 2. Functional analysis elucidating the top 10 skin functional categories for the affected genes in microarray.

Category	Number of Genes
Cellular protein metabolic process	192
Protein metabolic process	212
Cellular macromolecule metabolic process	237
Organonitrogen compound metabolic process	241
Symbiont process	63
Viral process	58
Interspecies interaction between organisms	64
Macromolecule localization	132
Response to stress	170
Protein localization	117

3.2. B. Skin Microbiome Study

Synbio® Skin Cream was applied daily at home for ten days by healthy volunteers, and the microbial DNA was collected before and after period of application, as detailed in "Clinical test protocol, skin sampling and lab process methodology for microbial DNA extraction" in Methods. 16S rRNA genes amplification and sequencing, targeting the V3 - V4 regions, and skin microbiota profiling was performed, as described in "Microbiological clinical test result data analysis" in Methods. The data analyses were conducted in a blinded fashion, as the data analyst had no taxonomic information about the tested probiotic preparation. The dual-index data set was preprocessed to analyze the microbiota taxonomic composition, taxonomy assignment of sequences was performed next, to generate phylum to genus level composition matrices as well as species identification where possible region as described in Methods. Ordination of the data, using nonmetric multidimensional scaling based on Bray-Curtis distances, shows an overall high similarity of the samples per individual (**Figure 2**). According to the pre-set analysis parameters, significant changes were observed in 20 ASVs following probiotics application. In total, three ASV's were increased after probiotic use (p-values: 0.036, 0.049 and 0.055), all of them 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). An ordination map of the detection of changes, using Bray-Curtis' distance is presented in **Figure 2** below. Skin microbiome diversity showed differences before and after application of the synbiotic cream, but for most individuals the change in diversity was not too disruptive.

3.3. Acne Evaluation Status Before vs. After Synbio, Pre and Probiotic Treatment—A Clinical Test Results

Unbalance microbiome is known to be involved in the appearance and severity of Acne vulgaris of skin, characterized by the appearance of comedones, papules, pustules, cysts and/or scars, located mostly on body regions with a higher number of sebaceous glands [19]. In physio-pathology, bacterial caused inflammation is one of the major symptoms of acne, related to the increased sebaceous secretion level, follicular hyperkeratosis with keratin and sebum accumulation in the follicle, and bacteria colonization and inflammation [20]. A standard cosmetic oil in water emulsion, formulated with 1% of Synbio, a bacillus spores-based pre + probiotic blend, supplied by HeiQ-Chrisal, and described in "Tested materials" in Methods, was applied daily at home on facial skin by 31 women volunteers, meeting the acne prone skin inclusion criteria of oily skin on the face, with a minimum sebumetric value of 120 μg/cm^2 on frontal area, and presenting dilated pores, lack of smoothness and softness in the facial skin, and with a mild to moderate IGA Score (Grade 2 - 3) proven by a dermatologist. The

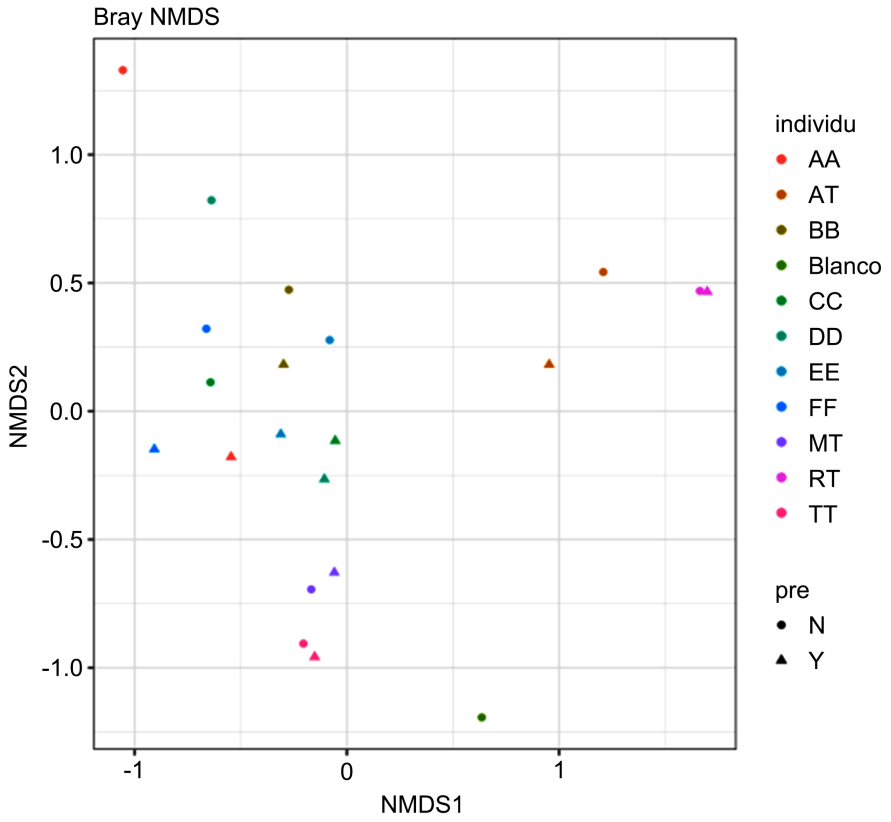

Figure 2. Data ordination map, using Bray-Curtis' distances to present skin microbiota profile modulation, following topical application of Synbio, Bacillus spores-based probiotic tested skincare preparation. Dots represent skin microbiota diversity before applying Synbio® skin cream. Triangles represent skin microbiota after Synbio® skin cream. Dots and triangles of the same color belong to the same test individual. The farther a dot and a triangle of the same color/individual are located, the bigger the influence of the synbiotic skin cream on the microbiome diversity of that individual.

clinical parameters, before and after treatments, were assessed, as detailed in "Acne evaluation status before vs. after pre and probiotic treatment—a clinical test" in Methods. A partial range of different skin status evaluation results is presented in **Figures 3-6** below:

Dermatologist Clinical Efficacy Assessment

A significant improvement of visual appearance of pores, skin smoothness and skin softness were observed after 1, 2 and 4 weeks of the application of the probiotic investigated product. IGA Score was used for the dermatologist assessment of Acne Severity, presenting a significant improvement on acne severity after 1, 2 and 4 weeks of product use as presented in **Figure 3**.

Oiliness Measurements with the device Sebumeter® SM 815

A significant reduction on skin oiliness was observed after 1 week and this low level remained on weeks 2 and 4 of investigational product use.

Count of Acne Lesions

Image Analyses was performed, using Visia® CR device, and a significant reduction of non-inflammatory lesions and total lesions was observed after 4 weeks

of investigational product use (data not shown). In addition, a significant improvement on skin redness was observed after 1 week of investigational product use (data not shown). A significant reduction of porphyrins was observed after 1, 2 and 4 weeks of investigational product use as presented in Figure 5.

Acne Quality of Life Index Questionnaire (Acne-QoL)

A subjective evaluation of the quality of life was performed by the Study Subjects. A significant improvement of the perception of quality of life of the study subjects was observed after 4 weeks of investigational product use as presented in Figure 6.

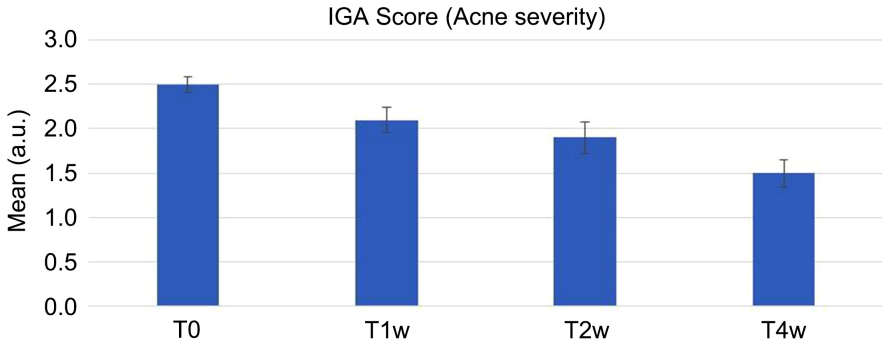

Figure 3. Dermatologist IGA score for Acne severity, means and standard error before treatment and after 1, 2 and 4 weeks of application.

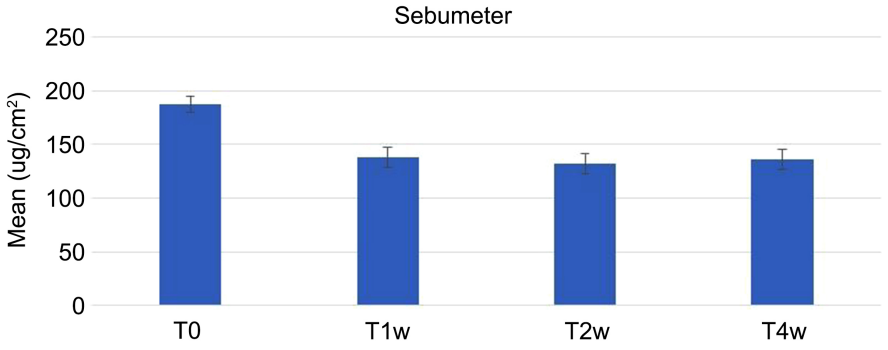

Figure 4. Sebumeter® SM 815 facial skin oiliness measurements, means and standard error before treatment and after 1, 2, and 4 weeks of application.

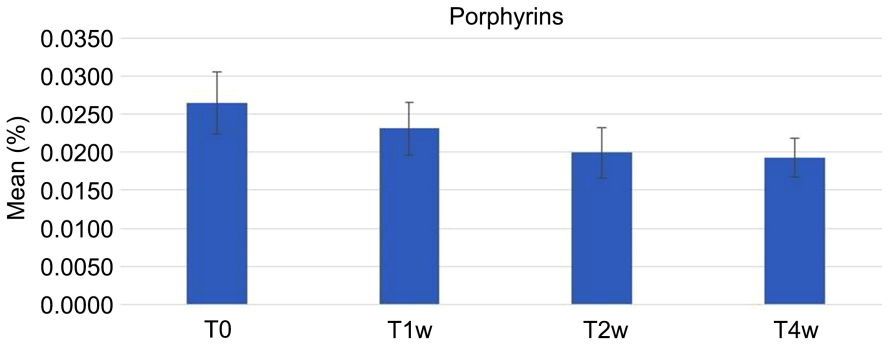

Figure 5. Visia® CR: image analyses for porphyrins, means and standard error before treatment and after 1, 2, and 4 weeks of application.

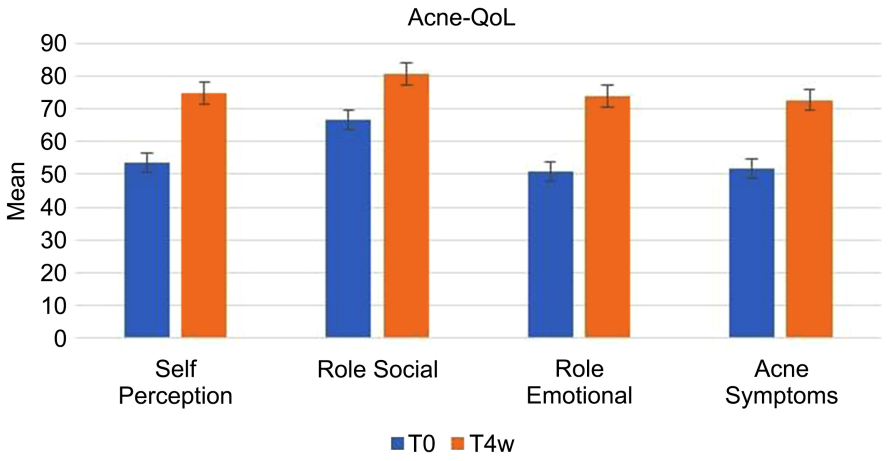

Figure 6. Acne Quality of Life Index: means and standard error of reported answers on questionnaires, performed by each participant before and at the end of the study.

4. Discussion

This paper evaluated the effect of several synbiotic preparations on human skin biology and the associated skin microbiome. Such potential effects were measured with a variety of analytical tools such as microarray, 16S gene sequencing and clinical skin analyses. Microarray is an efficient technology, enabling a parallel screening of modifications in the expression-level of human genes [21] [22]. Recent bioinformatic tools, allow processing of abundant microarray data, elucidating the involved biological pathways and affected functions [17] [18] [23]. The presented microarray results demonstrate the profound impact of topical application of Synbio preparations on gene expression. The expression level of more than 10% of 19,254 evaluated genes was affected. Furthermore, the significance of the transcriptome effect on skin biology was assured, via bioinformatic analysis, suggesting the involvement of key biological pathways and essential functions. Blending the synbiotic concentrate with Polygonum aviculare plant extract, known for its antioxidant effects, and Wnt/β-catenin activation based wound healing [13] [14], had triggered a distinct and relatively wide range of gene expression alterations. This result demonstrates the sensitivity of skin responses, when exposed to different active substances, lead to the activation of separate biological pathways. The opportunity to use gene expression techniques is raised in upcoming product development processes, enabling to targeting an accurate desired outcome. However, the transcriptome results, are limited to the gene expression level, and downstream levels, such as proteomics, must be further investigated, to well establish a specific biological effect.

The effectiveness of formulating live bacteria/probiotics in a leave-on skincare product is highly depends on the microorganism's capability to survive in the product during the product's shelf life [6] [7] [8]. Furthermore, the probiotics must also remain active on the skin upon product application, for a long enough time to exert beneficial effects. In a small scale "proof of concept" clinical test, a synbiotic skincare application, was shown to introduce live, active *Bacillus* pro-

biotics on the skin. After stopping the product application, the Bacillus applied strains were detectable for approximately 10 days. This test showed that it is possible to keep Bacillus probiotics alive in a skincare product and transfer them to the skin, where they remain active for a couple of days. We suppose that this significant surviving rate is partially achieved due to the presence of inulin in the synbiotic product. Inulin prebiotic sugar, is believed to boost and activate the latent Bacillus spores, speed up their germination process, and enhance the vegetative Bacillus cell to remain active for several days on the skin. Further research is needed to study the process of Bacillus spores' germination after topical application on skin surface.

By 16S DNA sequencing, the microbiome composition of 10 test persons was evaluated before and after the application of Synbio® skin cream. The data suggest the enrichment of Bacillus species, probably, due to the Bacillus species, loaded in the applied product. Furthermore, several microorganisms related to skin pathologies were reduced after the application of the synbiotic skin cream. Other skin microbiome species, representing a healthier microbiome state, were found to increase. These results may indicate that the application of a synbiotic skin cream can positively influence the skin microbiome within a short time frame. It is assumed that when the synbiotic product is applied frequently, a steady level of probiotics on the skin is stabilized, and the overall skin microbiome status is healthier. The realization of this promising assumption should be deeply investigated.

In addition, the results also show that the skin microbiome diversity of each test individual changed significantly, but not too drastically to cause a disruption of the original person's skin microbiome. We believe that a gentle, positive modulation of natural microbiome is the preferred targeted effect of any skin treatment. Further studies are required to investigate the different aspects of synbiotic microbiome modulation, and the actual health benefits that could be obtained on skin level.

This paper also presents the results of a number of clinical trials with test subjects on the indicative effect of synbiotic skin cream on several skin conditions. Unbalanced microbiome is involved in the pathogenicity of lots of skin diseases including acne vulgaris [19] [20]. Alteration of the local flora, with population expansion of Cutibacterium acnes (formerly: "Propionibacterium acnes"), is one of the major indications in acne physiopathology, engaged with increased level of sebaceous secretion, follicular hyperkeratosis, a keratin, and sebum accumulation in the follicle, boosting acne bacteria colonization, that may lead to skin inflammation [24]. Therefore, acne as an appropriate target for synbiotic treatment was suggested [25]. During a trial of four weeks, we assessed the efficacy of Synbio skin cream, topically applied on the face, to reduce acne symptoms, and improve skin status. Test results support anti-acne effects of the Synbio skin cream, including a significant reduction in IGA dermatologist score of acne severity, a diminution in the number of counted acne lesions, significant declines

in device measured skin oiliness and skin redness, visual improvement of the appearance of skin-pores, skin smoothness and softness, and improved Acne Quality of Life Index, recorded by patients, before and after Synbio application period. As such, applying synbiotic skin cream, if follows the experimental protocol, evidently had a beneficial effect on the test subject's skin acne condition.

Today, antimicrobial products are too often used in case of microbiology related skin conditions. Antimicrobial resistance is a growing concern to human health. Extensively administration of systemic antibiotics, had found to increase the percentage of developing pathogenic antimicrobial resistance [26]. Attenuation of the evolved risk of antimicrobial resistance is suggested. Daily application of synbiotic skincare as a proactive treat, may be considered in general, and particularly as a complementary or as an alternative to systemic antibiotic administration.

5. Conclusion

Topical application of a bacillus spore-based, pre and probiotic synbiotic preparation, activates a profound effect on skin biology, expressed in the transcriptome level. Despite the challenging environmental conditions, bacillus spores, when applied on human skin, successfully survive, and remain active. Bacillus probiotic activity is believed to participate in Synbio therapeutic effects on acne symptoms, a bacteria involved inflammatory skin disease. The deep influence of the synbiotic skincare, demonstrated in a clinical work, suggests its practice as an active player in future skin microbiome related treatments. Further research is needed to establish the efficacy of synbioic skincare application, to heal various unbalanced microbiome related skin conditions.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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