Active Beauty
Leadership in Microbiomics for Cosmetics

White Paper

Givaudan

engage your senses

1. "The"human microbiome

…or the human "microbiomes"

Human microbiome, previously referred to as human microflora, or human bacterial flora, has been known for decades. Microflora, or as we now call it more precisely **microbiota**, is in fact the assemblage of microorganisms present in a defined environment. Today what we name **microbiome** is a little bit more global, and refers to the microorganisms' community (the microbiota) plus their genes and their surrounding environment. Gathering pivotal information from the genes of a microbiota has been made possible by the tremendous development of genomic technologies over the last two decades, and in particular of a global analysis method called **metagenomics**, which allows studying all microbiota genes in one experience.[1]

The human microbiota is composed of a huge **diversity** of bacteria, yeasts, fungi and other viruses, and their total number is estimated to 10,000 different species.[2] However, the large majority of them are still unknown as they cannot be isolated and studied in laboratory conditions (90% of them at least!). In fact, most of them require very specific living conditions, and in general these are symbiotic conditions with many other microbial partners of the community.[3] Such complex and dynamic systems are very hard, or almost impossible, to reproduce in lab environments, although some models appear.[4]

But if the **complexity** of a bacterial community is intense, so is our body. As humans and microorganisms co-evolved for millions of years, microorganisms living in and on our body adapted to the variety of our organs. In fact bacterial communities are colonizing all of our pieces, including gut, mouth, nose, vagina, skin… and even blood. But as these organs provide various conditions for bacterial life - such as moisture or dryness, available nutrients, pH and temperature -, these environments have shaped a variety of species communities, each with typical and distinct phenotypes and abundance. This is the reason why, when we talk about human microbiome, we should say human microbiome**s**.

As an illustration, and this is surely one of the most famous ones, *Scientific American* edited in 2012 an interactive tool to explore the general patchwork of our microbiomes, showing which were the **most abundant microbial families** in every area of our body.[5]

In 2008, the National Institutes of Health started the Human Microbiome Project, designed to help understand the health implications of human bacterial flora. During 5 years, a network of not less than 200 researchers labored on deciphering our microbiomes and their link with our health. A total of 250 patents and 370 scientific papers were published, helping the scientific community to better understand our cohabitation with our microbes.

Key numbers arose from these studies, but also a deeper knowledge on **host-microbe interactions** in general, and in particular those related to diseases.[6] For instance, typical bacterial compositions have been identified between altered lung microbiome and cystic fibrosis, chronic obstructive pulmonary disease, or asthma, and between **unbalanced** intestinal tract microbiome and individuals with cystic fibrosis and diabetes.[7]

If interactions between microbial communities and host have been demonstrated in several

[1] Molecular biological access to the chemistry of unknown soil microbes: A new frontier for natural products. J Handelsman *et al*., *Chemistry & Biology* **1998**, 5, R245–R249
[2] Human Microbiome Project data, 2013
[3] The ecology of the microbiome: Networks, competition, and stability. KZ Coyte *et al*., *Science* **2015**, 350, 663-666
[4] *In vitro* models of the human microbiota and microbiome. JAK McDonald, *Emerging Topics in Life Sciences* **2017**, 1, 373-384
[5] https://www.scientificamerican.com/article/microbiome-graphic-explore-human-microbiome/
[6] The Human Microbiome: From Symbiosis to Pathogenesis. EA Eloe-Fadrosh and DA Rasko, *Annu Rev Med.* **2013**, 64, 145–163
[7] Computational Approaches to Study Microbes and Microbiomes. CS Greene *et al*., *Pac Symp Biocomput*. **2016**, 21, 557–567

cases, a new axis linking our gut (microbiota) and our brain becomes obvious. In particular, recent studies bring new evidences on the role of gut microbiota in autism spectrum disorders.[8,9] This, of course, paves the way to new treatments promises.

Even more recently, new results on the microbiome gut-brain axis have also shown that gut microbiota could play a role on our sense of smell,[10] when others are looking at the side of nose microbiome.[11]

In conclusion, we discovered during the last decades that not only almost every part of the human body is home to an entire ecosystem of thousands of microorganisms, but also that these were playing an important role in regulating many of our physiological (and psychological?) processes.

Most importantly, we also understood that, in addition to the crucial beneficial role of some defined microbial species that we try to favor using probiotic and prebiotic treatments,[12] it was the **preservation of the whole microbiome equilibrium** that was the primary key.

2. Skin microbiota

Among our various microbiomes, the skin microbiota, which at Givaudan we call the *Stratum microbium*™, is surely the most diverse. With an average surface of 1.8 m^2, our skin is indeed a vast continent with various climates and diversified countries. From deserts (forearms, calves) to jungles (armpits, scalp), our skin provides many niches with **different ecological conditions** (humidity, pH, available nutrients). But skin is also exposed depending on our own environment, lifestyle, and fashion style, to a large variety of **external parameters**, such as outside temperature, UV-exposure, pollution… and contact! This wide variety of skin ecosystems results in many differences in cutaneous microbial composition and abundance depending on the body area, and once again we should talk about skin microbiome**s**.[13,14]

One of the first descriptions of this **microbiome diversity** on our largest organ was published by Elizabeth Grice and Julia Segre in 2011.[15] Their famous illustration of topographical distribution of bacteria on various skin sites is very explicit, but in fact represents microbial diversity on one individual only.

Later, studies on the skin microbiome have all shown that, if you compare volunteers within a panel by skin zone-to-skin zone, **we are all different**. In fact, our skin microbiome is first determined by early microbial colonization right after birth (and delivery type has its impact[16]), but is then matured with increasing microbial diversity by our life environment, especially during the first year of life.[17] On this point, a study performed in 2015 on Korean twins tried to explore genetic and environmental effects on skin microbiome modulations.[18]

Today, the diversity of skin-associated bacterial communities among people is more and more considered for forensics identification, with different skin microbiome profiling methods being developed.[19,20,21]

[8] The Gut Microbiota and Autism Spectrum Disorders. Q Li *et al*., *Front Cell Neurosci*. **2017**, 11, 120
[9] New evidences on the altered gut microbiota in autism spectrum disorders. F Strati *et al*., *Microbiome* **2017**, 5, 24
[10] Disruptive physiology: olfaction and the microbiome-gut-brain axis. J Bienenstock *et al*., *Biol Rev Camb Philos Soc*. **2018**, 93, 390-403
[11] The nasal microbiome mirrors and potentially shapes olfactory function. K Koskinen *et al*., *Scientific Reports* **2018**, 8, 1296
[12] Probiotics, prebiotics and synbiotics- a review. KR Pandey *et al*., *J Food Sci Technol*. **2015**, 52, 7577–7587
[13] Structure and function of the human skin microbiome. NN Schommer and RL Gallo, *Trends Microbiol*. **2013**, 21, 660-668
[14] The human skin microbiome. AL Byrd *et al*., *Nat Rev Microbiol*. **2018**, doi:10.1038/nrmicro.2017.157
[15] The skin microbiome. EA Grice and JA Segre, *Nature Reviews Microbiology* **2011**, 9, 244-253
[16] Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. MG Dominguez-Bello *et al*., *PNAS* **2010**, 107, 11971-11975
[17] Diversity of the Human Skin Microbiome Early in Life. KA Capone *et al*., *J. of Inv. Dermatol*. **2011**, 131, 2026–2032
[18] Genetic associations and shared environmental effects on the skin microbiome of Korean twins. J Si *et al*., *BMC Genomics* **2015**, 16, 992
[19] Integrating the microbiome as a resource in the forensics toolkit. TH Clarke *et al*., *Forensic Science International: Genetics* **2017**, 30, 141-147
[20] The human microbiome: an emerging tool in forensics. JT Hampton-Marcell *et al*., *Microb Biotechnol*. **2017**, 10, 228-230
[21] Forensic human identification using skin microbiomes. SE Schmedes *et al*., *Appl Environ Microbiol*. **2017**, 83, e01672-17

Studies also looked at demonstrating the impact of skin contact with other people - or animals! - on the microbiota composition. For instance, James Meadow and co-workers showed in 2013 the influence of a contact sport (roller derby in this case) on the modulation of skin microbial composition upon crossed bouts.[22] Skin microbiome profiles of all players tended to overlap after tournament.

At the same time, Se Jin Song *et al*. showed the influence, on human skin microbial communities, of having a dog at home.[23] They measured in particular that dog-ownership significantly increased the shared skin microbiota in cohabiting adults, and dog-owning adults shared more skin microbiota with their own dogs than with other dogs. Their results suggest that direct and frequent contact with our cohabitants (humans and pets) may significantly shape the composition of our skin microbial communities.

But microbes on our skin also communicate with our body,[24] and in particular play an important role in our immune system, as was described by Julia Segre (see figure below).[25] As a prolific expert in the field, Richard Gallo also demonstrated the link between cutaneous microbiota and skin health.[26]

In fact, if most of the microbiome research is focused on the gut (skin microbiome scientific papers represent less than 4% of the total microbiome studies published every year, according to PubMed), knowledge on the association of skin disorders and the presence of typical microbial species is steadily increasing. It has to be noticed that microbial communities from the skin differs strongly from the microbiomes of other organs, as was measured by The Human Microbiome Project Consortium,[27] and that it is furthermore constantly subject to external influences.

But despite this complexity and the lack of strong population surveys and skin epidemiologic studies, it has become clear that several skin disorders were correlated with alterations in microbial communities, where differences in the microbes present in diseased skin versus those present in healthy skin have been described. However, it is still not clear whether microbiome imbalance leads to skin pathologies, or whether underlying skin conditions result in a modification of microbial equilibrium.

Among the different correlations that have been identified and demonstrated, one can mention:
- atopic dermatitis with *Staphylococcus aureus* species (and a low-diversity skin microbiota),[28,29]
- acne with *Cutibacterium acnes* and *Staphylococcus*,[30] but also with a general imbalance of skin microbiota,[31]
- dandruff with *Malassezia* species and a disrupted balance between bacteria and fungi,[32,33,34] or
- psoriasis with an increase in Firmicutes (mainly *Streptococcus* and *Staphylococcus*) and Actinobacteria (mainly *Corynebacterium* and *Pro-*

[22] Significant changes in the skin microbiome mediated by the sport of roller derby. JF Meadow *et al*., *PeerJ* **2013**, 1:e53
[23] Cohabiting family members share microbiota with one another and with their dogs. SJ Song *et al*., *eLife* **2013**, 2:e00458
[24] Skin microbiota-host interactions. YE Chen *et al*., *Nature* **2018**, 553, 427-436
[25] Dialogue between skin microbiota and immunity. Y Belkaid and JA Segre, *Science* **2014**, 346, 954-959
[26] Functions of the skin microbiota in health and disease. JA Sanford and RL Gallo, *Seminars in Immunology* **2013**, 25, 370-377

[27] Structure, function and diversity of the healthy human microbiome. HMC, *Nature* **2012**, 486, 207-214
[28] Microbiome in atopic dermatitis. U Wollina, *Clinical, Cosmetic and Investigational Dermatology* **2017**,10, 51-56
[29] The role of the skin microbiome in atopic dermatitis: a systematic review. RD Bjerre *et al*., *British Journal of Dermatology* **2017**, 177, 1272–1278
[30] Skin microbiome and acne vulgaris: Staphylococcus, a new actor in acne. B Dreno *et al*., *Experimental Dermatology* **2017**, 26, 798-803
[31] The balance of metagenomic elements shapes the skin microbiome in acne and health. E Barnard *et al*., *Scientific Reports* **2016**, 6:39491
[32] Characterization of the major bacterial–fungal populations colonizing dandruff scalps in Shanghai, China, shows microbial disequilibrium. L Wang *et al*., *Experimental Dermatology* **2015**, 24, 381-400
[33] Dandruff is associated with the conjoined interactions between host and microorganisms. Z Xu *et al*., *Scientific Reports* **2016**, 6:24877
[34] Collapse of human scalp microbiome network in dandruff and seborrheic dermatitis. T Park *et al*., *Experimental Dermatology* **2017**, 26, 835-838

pionibacterium),[35] but above all linked with a lower microbial diversity.[36,37]

Once again, it is the **high diversity** of the microbiota and the **protection of its balance** that prevails for a healthy skin microbiota.

3. Cosmetics and skin microflora

One of an additional role of microbes living on our skin is the production, or modulation, of body odors, in particular to produce axillary malodors by decomposition of apocrine sweat (usually by Corynebacteria), or foot odors from eccrine sweat.[38,39]
In fact skin microbiota participates in the global olfactory "signature" of human skin. In 1953 first studies demonstrated the role of bacteria in the axillary odor formation,[40] later followed by the work of famous dermatologist Albert Kligman.[41,42]

Deodorants were thus the first cosmetic products developed to have an effect on our skin microbiota, with early studies on the effect of topical antibacterial agents on the axillary microbiome in 1963.[43]
These discoveries were soon further exploited by Albert Kligman to develop new approaches to treat acne, while he coined the term "cosmeceuticals".[44] In addition, he developed new therapies to treat atopic dermatitis or rosacea, or even dandruff.[45,46]
Today research on the axillary bacterial community and deodorants is still pursued, taking benefit of new technologies and regularly leading to better knowledge of the biochemical processes to malodors formation.[47]

On this point, microbiota transplant that is operated from a healthy individual into a recipient to treat gastrointestinal diseases has inspired a much more disruptive approach to fight against armpit malodors.[48]
Indeed its inventor, Chris Callewaert, experienced a successful, though of non-lasting effect, transplant of the skin microbiome from a 'non-smelly' donor to a 'smelly' acceptor, hence eradicating malodors. Still working on improving his approach, Chris Callewaert has since created "DrArmpit", a science communication platform where scientific research about armpit microbiology in relation to body odor is made available to the public.

Deep knowledge on gut microbiota functioning and in particular its beneficial modulation upon probiotics or prebiotics ingredients supply has served the cosmetic industry. Several products are available on the market, and the logic is simple: microorganisms that are good for our gut cannot be bad for our skin.

Probiotics are living microorganisms that are supplied to the gut (via ingestion) to rebalance an altered microbiome and modify its equilibrium towards a more healthy one. These are the classical *Bifidobacterium* or *Lactobacillus* we can find in yoghurt, for instance. **Prebiotics**, meanwhile, are food ingredients that promote the growth of beneficial microorganisms in the intestines.

[35] Community differentiation of the cutaneous microbiota in psoriasis. AV Alekseyenko *et al*., *Microbiome* **2013**, 1:31
[36] Unexplored diversity and strain-level structure of the skin microbiome associated with psoriasis. A Tett *et al*., *npj Biofilms and Microbiomes* **2017**, 3:14
[37] Skin Microbiome: An Actor in the Pathogenesis of Psoriasis. WM Wang and HZ Jin, *Chinese Medical Journal* **2018**, 131, 95-98
[38] Microbial flora and odor of the healthy human skin. HC Korting, *Hautarzt*. **1988**, 39, 564-568
[39] Mapping axillary microbiota responsible for body odours using a culture-independent approach. M Troccaz *et al*., *Microbiome* **2015**, 3:3
[40] Axillary odor; experimental study of the role of bacteria, apocrine sweat, and deodorants. WB Shelley *et al*., *AMA Arch Derm Syphilol*. **1953**, 68, 430-446
[41] The bacteria responsible for apocrine odor. AM Kligman and JS Strauss, *J Invest Dermatol*. **1956**, 27, 67-71
[42] The microbiology of the human axilla and its relationship to axillary odor. JJ Leyden *et al*., *J Invest Dermatol*. **1981**, 77, 413-416
[43] The effect of topical antibacterial agents on the bacterial flora of the axilla. NH Shehadeh and AM Kligman, *J Invest Dermatol*. **1963**, 40, 61-71
[44] Control of free fatty acids in human surface lipids by Corynebacterium acnes. RR Marples *et al*., *J Invest Dermatol*. **1971**, 56, 127-131
[45] Methods for evaluating topical antibacterial agents on human skin. RR Marples and AM Kligman, *Antimicrob Agents Chemother*. **1974**, 5, 323-329
[46] Role of microorganisms in dandruff. . JJ Leyden *et al*., *Arch Dermatol*. **1976**, 112, 333-338
[47] Deodorants and antiperspirants affect the axillary bacterial community. C Callewaert *et al*., *Arch Dermatol Res*. **2014**, 306, 701-710
[48] Towards a bacterial treatment for armpit malodour. C Callewaert *et al*., *Experimental Dermatology*. **2017**, 26, 388–391

Probiotics and prebiotics can provide beneficial effects to the skin as these are targeted to favor those cutaneous microbial species which are believed to be 'good' for our skin. In both cases, the intention is to balance skin microbiota to enhance it.
However, since probiotics are living bacteria, there are quite a few hurdles before integrating them into our skin microbiome. Maintaining their integrity and survival in the cosmetic formulation is the first one, while the second is their lifetime and impact into the cutaneous microbiota itself, as gut and skin ecosystems are highly different. Thus, 'probiotics'-containing beauty products are generally actually prebiotics, composed either of **probiotics lysates** or of **ferment extracts**.

In that field, **communication to the consumers** is key, and comes with their **education**. In fact, skin microbiome communication is booming in beauty magazines and professional press over the last two years, and blogs are flourishing talking about those little microbes that are best allies for our beauty.

For instance Elle Magazine, in 2015, named the probiotics as the "**bacteria which makes us beautiful**". One year after, Vogue claimed probiotics an "**invisible anti-aging shield**", which "by strengthening the skin microflora, also inhibit the ageing signs in formation", and was followed by StyleCaster. While Fashionista encouraged to "start working probiotics into skincare routine". In 2017, Elle Magazine, Madame Figaro, Shape and Vanity Fair all talked about probiotics and/or skin microbiome as being the new, unavoidable, **solution for beauty**. The Atlantic,[49] The New York Times,[50] Vanity Fair,[51] and very recently Forbes,[52] also dedicated at least an article on the subject.

As a summary, new cosmetic claims appear that are based on these microbiome ingredients, related to Age-Defying, New-Age, Healthy Hands, Detox Gentle, Rebalancing Masks, Probiotic or Biome-friendly.

4. Leading the way in skin microbiomics

Skin microbiome is, without contest, a full player in our beauty and well-being. But how do we assess it, how do we evaluate the impact of a cosmetic ingredient or end-product on the *Stratum microbium*™, and how do we demonstrate that its population is well balanced?

As we have seen it previously, most of the microbes living on our skin cannot be isolated and characterized under lab conditions, and therefore one has to target the entire genes set of the *Stratum microbium*™ to get the most of it. However, as genes from one species cannot be separated from the ones of its neighbour, a global approach called metagenomics was developed in the late 90s.[1]

Metagenomics is a very powerful tool, but with a lot of potential pitfalls in study design, microbial DNA extraction and preparation, or sequences analysis for instance. As a "multi(tricky)step" procedure, precision, care and rigor are obligatory.

Metagenomics can be conducted via 2 different pathways, depending on your needs to be descriptive only (taxonomic profiling down to the genus, done from 16S ribosomal RNA analysis), or your wish to assess the functional potential and a detailed composition of your microbiome (done using whole genome sequencing techniques).[53]

The different steps of such a taxonomic profiling study (the simplest of both approaches) have been nicely described by Heidi Kong and co-

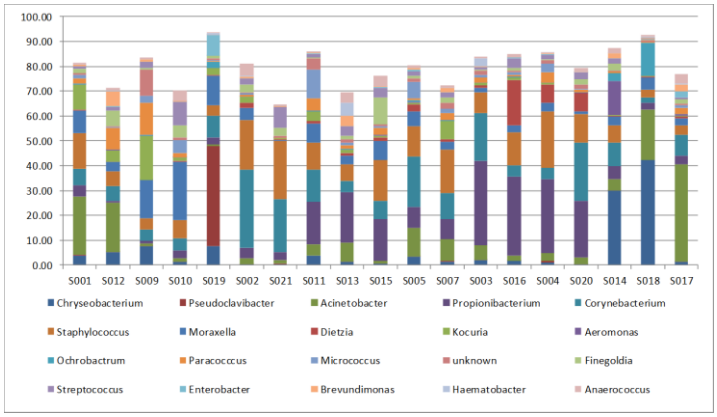
[49] What Is the Right Way to Wash Your Hands? S Zhang, *The Atlantic* Jan 23, **2017**
[50] Microbes, a Love Story. M Velasquez-Manoff, *The New York Times* Feb 10, **2017**
[51] 30 millions d'amies (au cm^2). *Vanity Fair* Oct 20, **2017**
[52] Why Probiotic Beauty Products Are Great For Your Skin. C Shatzman, *Forbes* Jan 8, **2018**

[53] Genomic approaches to studying the human microbiota. GM Weinstock, *Nature* **2012**, 489, 250-256

workers, in particular using the following scheme.[54]

Going for a metagenomic 16S ribosomal RNA analysis (or "**16S sequencing**") has become usual, as the techniques matured and the services laboratories developed. The method, which is instructive enough to compare microbiome taxonomic profiles (on the basis of microorganisms relative abundances), is nevertheless **limited to bacteria**. Thus, no information on other members of the microbiome – such as yeasts or fungi for instance -, will be gathered, and relative abundances will be calculated only on the basis of bacterial families.
In addition, 16S sequencing suffers from two constraints: the first is its lack of phylogenetic precision (taxonomic profiles can only be significatively estimated at the **genus level minimum**), and the second is its dependence to the variable region of the 16S rRNA gene that is used. Consequently, microbiome data have to be compared, from one study to the other, only if the procedure has been the same. Finally, PCR amplification of microbial DNA (to obtain enough material for sequencing) is often subject to bias from the choice of the primers.

As a conclusion, typical taxonomic profiles (bacterial genera relative abundance) that can be obtained are shown in the following figure (unpublished results).



Diversity in genus profiles of forearms skin microbiota from 19 volunteers, as obtained from a 16S ribosomal RNA analysis (each color refers to a different genus, with relative abundance on the total bacterial content)

While 16S sequencing provides a good estimate of who is there, the resulting data does not inform about any functional potential. To obtain such an information, and in addition to get an exhaustive (bacteria, fungi, yeasts…) description of the microbiota population down to the species (and sometimes the strains), one has to move towards **whole genome sequencing** (**WGS**), also called "shotgun sequencing".

In WGS sequencing all the pieces of DNA within a sample are cut up into small pieces, and all gene fragments are sequenced without any further selection or treatment. Once sequences data have been collected, computer analysis allows recreating the entire genome of all the organisms present in a sample. Gene fragments sequences are usually compared and assigned by alignment with databases of genetic information, and phylogenetic affiliation is compiled, including for unknown species. Finally, as all DNA information is decoded (and not only a fragment of the 16S gene), enzymes present in these microorganisms are identified at the same time, revealing the functional potential of the microbiota.

XC Morgan and C Huttenhower, in particular, have compared the two metagenomic approaches, and designed the following comparative process scheme.[55]

At Givaudan Active Beauty we master both techniques equally, with a **particular know-how on cutaneous microbiota sample col-**

[54] Performing skin microbiome research: A method to the madness. HH Kong *et al.*, *J Invest Dermatol*. **2017**, 137, 561-568

[55] Chapter 12: Human Microbiome Analysis. XC Morgan and C Huttenhower, *PLoS Comput Biol* **2012**, 8, e1002808

lection, as well as proprietary genes databases that are exploited with home-made bioinformatics pipelines. Indeed, after many years spent in developing procedures and databases to explore soil and gut microbiomes, we focused on skin metagenomics techniques and were one of the first to publish on this very specific topic.[56,57]

Today we associate the use of **genomic technologies** with **classical microbiology** to explore the *Stratum microbium*™ of various skin regions, including the face (cheeks, forehead, and nose), scalp, arms, armpits, and legs. In addition to these microbiomics techniques, we also develop metatranscriptomics protocols, with the aim to characterize the composition and functionality of complex microbial communities.

As such, we are the only fragrance house, and supplier of cosmetic ingredients, with integrated expertise and its own **Applied Microbiomics Centre of Excellence** (which is located in Toulouse, France).

5. Stratum Microbium™ : Microbiomics and cosmetics

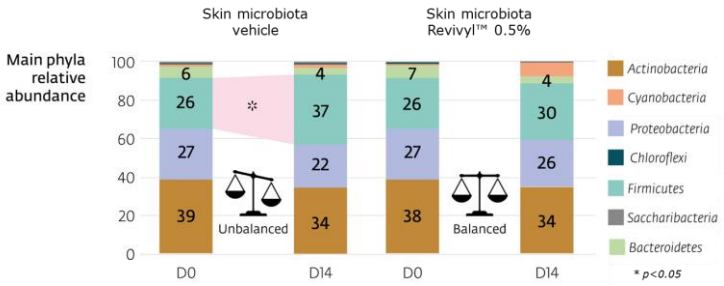
As experts of skin microbiomics, we are dedicated to the development of new cosmetic ingredients linked with the *Stratum microbium*™. This exclusive knowledge enables us to understand cosmetic ingredients effect on skin microbiota, to design new ingredients acting directly on skin microbiota, and ultimately, to link skin microbiota composition to beauty.

As seen before, the overwhelming majority of products available on the market in this category are made of prebiotics, with a few of them containing probiotics. At Givaudan Active Beauty we also offer 2 products in that frame, in an approach that we name **Balance to Enhance**.

And to this skin microbiota leveraging range, we add 2 unique consumers' driven approaches, which we call **Protect to Care** and **Trigger to Activate**.

The first concept is based on four observations: (i) our skin microbiota is beneficial for our beauty and well-being when it is well balanced, with a high microbial diversity; (ii) with the absence of any skin disorder, our current *Stratum microbium*™ represents our own healthy microbiome; (iii) applying any personal care products on our skin, including a prebiotic formula, could lead to a disturbance of our microbiome; and (iv) **we are all unique**.

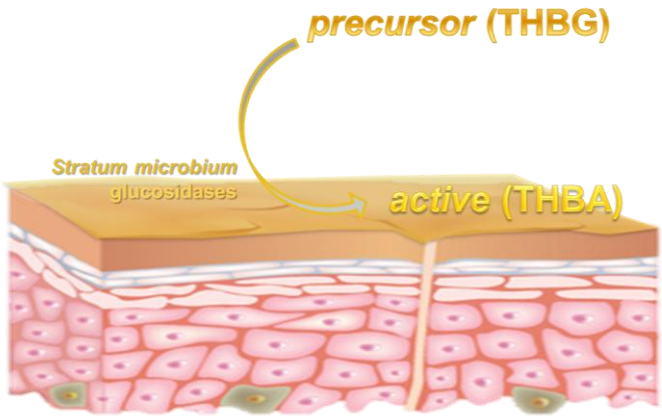
Thus, a new ingredient, called Revivyl™ and developed for skin renewal activation, was shown to counteract the microbiome disturbance induced by the cosmetic base itself. In a way to **Care** of ourselves, Revivyl™ **Protects** our skin microbiome from external disturbance. This protection effect was demonstrated via a comparative 16S sequencing study, performed on the forearms of a panel of 19 women volunteers. After 2 weeks of treatment, Revivyl™ was shown to maintain the initial balance of the cutaneous microbial population, while a significant imbalance was observed by using the cosmetic vehicle only (see figure).



Protection of the skin microbiota balance using Revivyl™ (in this case, protects skin microflora equilibrium from an imbalance in Firmicutes provoked by the cosmetic base without Revivyl™)

[56] Life on Human Surfaces: Skin Metagenomics. A Mathieu *et al*., *PLoS ONE* **2013**, 8, e65288
[57] The future of skin metagenomics. A Mathieu *et al*., *Research in Microbiology* **2014**, 165, 69e76

As a second unique alternative approach for skin microbiota leveraging products, we developed a strategy to **Trigger** the skin microbiome intrinsic capabilities to **Activate** ingredients and release their benefits. This is a precursor approach, in which the precursor, stable but inactive, is transformed by specific enzymes present in the cutaneous microbial community into an active ingredient, as illustrated in the following scheme.



In the case of our Brightenyl™ ingredient, launched in 2015 for skin brightening, redness decrease and pore size reduction applications, the required enzymes are alpha-glucosidases. Their presence was demonstrated by 2 parallel studies: a first incubation of Brightenyl™ with skin microbiota samples collected from the cheeks, forehead, nose, and forearms of 9 volunteers, and monitoring of precursor and active compounds using HPLC analysis. This showed the biological transformation of the inactive precursor into the desired active compound. And a second study done using Whole Genome Sequencing of DNA extracted from forehead microbiota samples collected on 2 volunteers, and which demonstrated the presence of several alpha-glucosidase genes to common bacterial species found on human skin (see table below for the most representative species).

Bacterial identification	Occurrence	Identity
Propionibacterium acnes	28	75-100%
Staphylococcus epidermidis	17	99-100%
Staphylococcus hominis	15	95-100%
Staphylococcus capitis	9	98-100%
Staphylococcus sp. AL1	8	99-100%
Staphylococcus warneri	8	99%
Staphylococcus haemolyticus	7	96-97%
Propionibacterium sp. HGH0353	6	87-99%
Propionibacterium acnes (HL103PA1)	5	75-100%
Propionibacterium acnes (JCM 18918)	5	99-100%
Propionibacterium sp. KPL2008	5	69-100%
Macrococcus caseolyticus	4	75-89%
Micrococcus luteus	4	70-99%
Propionibacterium granulosum	4	85-96%
Dermacoccus sp. Ellin185	3	93-95%

With a similar approach based on the needs for enzymatic (esterolytic) activation of an inactive precursor, Questice® completes our range of **Trigger to Activate** approach ingredients.

With currently almost 1 trillion of microbiome data and solid expertise and know-how, we master the whole research process for microbiomics, from the swabbing to the DNA extraction and sequencing until the bioinformatics data analysis. Givaudan Active Beauty continues to develop active cosmetic ingredients, designed to activate, protect or balance the skin microbiota. Indeed, as we are all unique, future ingredients and end-products shall adapt to the uniqueness of their consumers and their microbial ecosystem.

In particular, future ingredients shall be designed to counteract aging physiological changes, which also impact skin microbial diversity. Recent studies showed, for example, a shift in skin microbiome composition correlated with chronological and physiological skin aging factors.[58,59] Differences in aging skin microbiome are also observed between men and women, with variability depending upon the body area.

[58] Aging-related changes in the diversity of women's skin microbiomes associated with oral bacteria. N Shibagaki *et al*., *Scientific Reports* **2017**, 7:10567
[59] The microbiota and microbiome in aging: potential implications in health and age-related diseases. HJ Zapata and VJ Quagliarello, *J Am Geriatr Soc*. **2015**, 63, 776-781

Looking more closely at certain species such as Archaea, Christine Moissl-Eichinger *et al*. revealed that archaea are more abundant in human subjects either older than 60 years or younger than 12 years as compared to middle-aged human subjects.[60] This could open up the way to developing new anti-aging products based on archaeal signatures.
Finally, a very recent press release (Nov 30, 2017) revealed the discovery, by Amway in collaboration with Microbiome Insights, of two *Corynebacteria* species - one associated with younger people and the other with older people. The 'old skin' *Corynebacteria* species tend to displace the other during middle age (40-49). Interestingly, the two bacteria were found to be co-exclusive meaning they did not exist simultaneously at the same skin site. And furthermore, in addition to being associated with chronological age, the 'old skin' *Corynebacterium* was found to be associated with skin redness, wrinkles and age spots, suggesting it could be a target for treatment intervention.

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