Empowering the Micro-World of the Skin Microbiota:

Approaches to Maintain Nature's Ideal Homeostasis for Betterment of Cosmetic Products

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Editor's Note: There is a lot being said about the microbiome these days. This article will stand with the best of them. Enjoy!

Abstract

Skin is a complex environment where billions of microorganisms live providing a unique environment for each host, collectively referred to as the skin micriobiota. Skin microbiota is, therefore, the result of an equilibrium between protective and pathogens species of those microorganisms.

However, this balance can be disrupted by stressors. The alteration of skin microbiota, known as dysbiosis, has been associated with skin disorders. This article is designed to demonstrate different approaches to the prevention of skin microbiota dysbiosis.

Skin definition, composition and further insights

The skin is the largest organ of the human body in surface and weight, serving as a physical barrier which protects the body from external aggression. An adult's skin hosts an average population of 1,000 billion microorganisms among fungi, viruses and bacteria [1, 2, 3]. This fauna lives and is mobile on the surface as well as in the superficial layers of the epidermis to down to the hair follicles and glands. The fauna forming a complex ecosystem collectively referred to as skin microbiota. This tiny, but important Micro-World is essential for the skin to main a "happy", balanced homeostastis barrier.

To environmental insults- thereby , accomplishing its mission of protection, immunity and defense.

Each of us has a distinctive combination of microorganisms all over our body, although scientists point out that skin microbiome varies a lot during our lives. This variation is linked to age, changes of lifestyle and to the external stressors we are submitted to [4]. Different body sites can also have completely different skin microbiota configurations, both inter- and intra-personally, linked to

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the peculiar characteristics of that precise micro-environment. For example, just focusing on the face, studies show that there are great differences between forehead and cheek skin microbiota, due to the existence of moist, dry and sebaceous skin sites[5].

Despite continuous changes in its composition, when the body is in a homeostatic status, skin microbiota seems to be an equilibrium between protective and pathogens microorganisms. These live together in a complex community and have a number of different symbiotic interactions. If we consider bacteria, the most important and frequent phyla living on human skin are Actinobacteria, Firmicutes, Proteobacteria and Bacteroidetes, without huge differences among ethnicities. Further, looking more deeply into more specific taxonomic classification, as class or genus or species, we can find differences among peoples' microbiomes even by looking at subjects with very similar age, lifestyle, and from the same ethnicities. The general truth for everyone's healthy condition seems to be the homeostasis of skin microbiota with its singular peculiarities. However, life being what it is, everyone's microbiota balance can be disrupted by external and internal stressors. A strong change in the environment, for example, can alter skin microbiota leading to its dysbiosis. This condition has been often associated with skin disorders (skin inflammation, sunburn, dandruff, atopic dermatitis, psoriasis, acne). To avoid microbiota dysbiosis, (i.e. maintaining microbiota equilibrium),we may prevent certain skin disorders.

Moreover, it is essential to maintain the skin microbiota's natural biodiversity in different body sites, in order to let the skin barrier work for us in the best way possible. Starting from these two fixed points, the development of ingredients that can naturally balance the skin microbiota, while not interacting with its physiological changes and its biodiversity, becomes essential for cosmetic innovation, in order to obtain optimal performance with the lowest impact on skin homeostasis.

Innovative, Performing and Efficient Ingredients for Health & Personal Care

ROELMI HPC has been working for more than 10 years on biofermentation technology, focusing on production of different proprietary probiotic strains, under the tradename Synbalance®. Every strain investigated has shown physiological peculiarity *in-vivo,* allowing a precise targeting of the various body axis by using different bacteria. Specific strains from Lactobacilli and Bifidobacteria genera have been selected to modulate the gut-skin axis: taken as food supplement they have shown a decrease of skin inflammation and consequently an increase of skin beauty. Nutraceutical effect for cosmetic benefit. With this axis approach, the activity of probiotics has been investigated on cognitive function, by looking at the gut-brain axis, for stress status modulation and memory performances.

ROELMI HPC made a step forward, realizing probiotics for cosmetic applications. A line of inactivated probiotics called ProbiOFF has been standardized thanks to heat technology after bacterial fermentation. By means of this approach, probiotics are no longer living cells. However, they still maintain their mimetic effect on the skin immune system since they do not lose their cell shape. It means that the body is able to recognize them as bacteria, as cells are dead but not destroyed like in lysate ingredients, so skin natural defense are activated by bacteria natural shape. ProbiOFF can be considered as prebiotics, as they become food for skin resident microbiota and an alert for human cells defense mechanisms. *In-vitro* data show inactivated probiotics have the capacity to inhibit inflammatory mediators by their direct interaction with immune cells.

Moreover, ROELMI HPC has focused its activity on postbiotics produced by fermentation, with a targeted effect on microbiota equilibrium. A powerful pure molecule able to rebalance the skin microenvironment against external stressors has also been developed and it demonstrates a consequent peculiar protection activity of Microbiota Biodiversity, whatever species composition it is in different people. This ingredient, marketed under the trade name of ÆCTive®, acts as a promoter of healthier skin by regulating skin homeostasis. Skin moisturization, elasticity and the general skin profilometry are seen to be improved thanks to a better skin microbiome equilibrium. This new development has been studied *in-vivo* by looking at the metagenomics of the skin microbiome and skin dermatological parameters. Volunteers belonging to both European and Asian *ethnicities* have been selected to globally enlarge ROELMI HPC expertise on this leading-edge topic arising from the understanding that the balance of skin microbiome species is essential for the good function of every kind of skin.

Finally, a considerable amount of work is ongoing in ROELMI HPC continues to expand research on bio-balancers that are molecules which can preserve finished cosmetic formulas with a low impact effect on skin microbiota equilibrium.

Focus on a new active ingredient for skin microbiota protection

As a new frontier of development, ROELMI HPC decided to focus on postbiotics, deepening its knowledge at the microbiome genomic level, and moving from healthy to unhealthy skin, where microbiome dysbiosis is the starting point.

ROELMI HPC, Italian producer of active & functional ingredients for Health & Personal Care markets, has developed a patented fermentation process based on a pure molecule which is naturally found in extremophilic microorganisms living in strong environmental conditions. Ectoine is known worldwide as an amino acid derivative. It allows bacteria that produce it to equilibrate the surrounding environment and protect it from external stresses (i.e. strong UV rays, chemical agents …). Ectoine is an osmolyte firstly isolated by Galinski and colleagues from the bacterium *Ectothiorhodospira halochloris* found in Wadi Natrun (Egypt), that mainly balances the extracellular environment salt concentration[6].

Starting from this great innovation of Nature, ROELMI HPC has standardized and industrialized the production of this molecule by fermentation thereby obtaining a balancer of the skin microenvironment that allows an equilibrium of skin microbiota living in specific sites. Moving from the Nature to our skin, the rebalance of external environmental aggression makes the skin a more comfortable place for the microbiota itself to live in, thereby maintaining its natural homeostasis and composition. As shown above, an equilibrated skin microbiota is also a prevention of potential skin disorders and a better condition for the skin for its normal function. ÆCTive® (INCI Ectoin) from ROELMI HPC is a pure molecule, from a non GMO bacterial strain, showing peculiar activity against environmental stresses. This active ingredient is ideal for skin microbiota to survive, proliferate and protect the skin helping cell to recover from acute stress.

Apart from the singular human microbiota composition, this innovation is effective on whatever skin type, as its first target is the skin microenvironment and not the single bacterial species.

The ingredient is, therefore, the guardian of optimal skin complexion by protecting the balance of everyone's Finger-Printed Microbiota Biodiversity.

ROELMI HPC has a pending patent on Ectoine application for the prevention of skin microbiota dysbiosis (EP18207080).

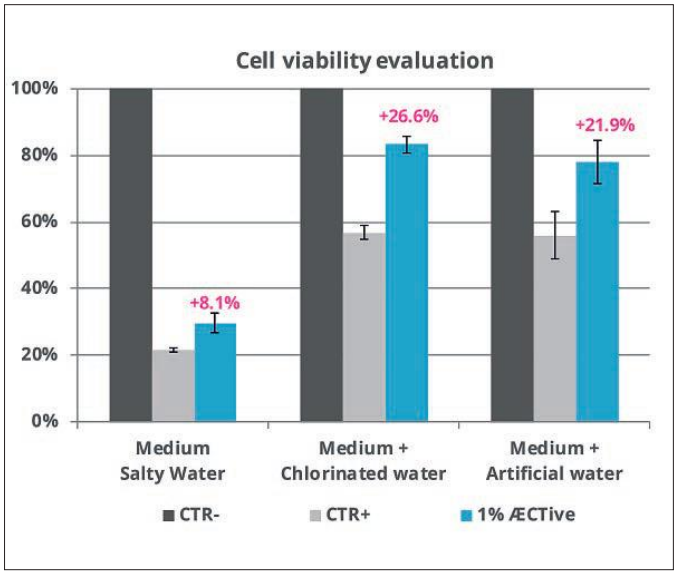
Development: Evidence of efficacy

The capability of the cosmetic active to contribute to the maintenance of cell homeostasis (cell viability and cell metabolism) after osmostress induction, so changing of environment equilibrium, has been evaluated by an *in-vitro* test. Human skin keratinocytes were treated with the cosmetic active at 3 concentrations (chosen after a preliminary cytotoxicity test). Different osmostress condition has been induced:

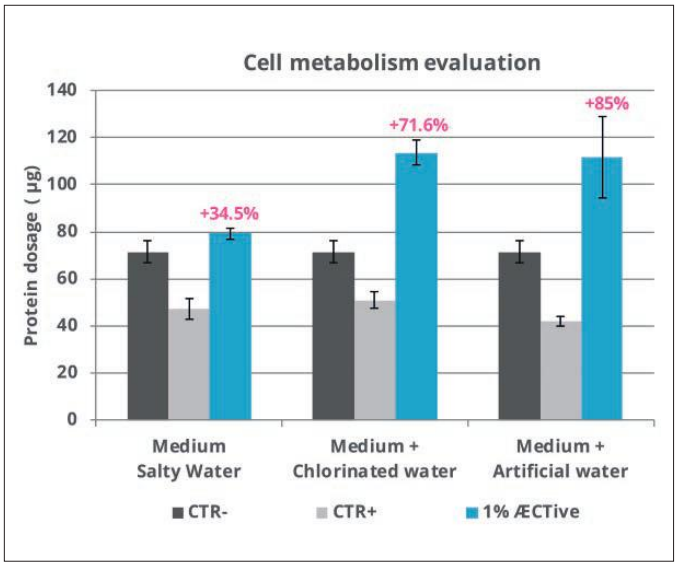
- by adding salt to the cell culture medium (to simulate marine environment)
- by adding chlorine to the cell culture medium (to simulate swimming pool environment)
- by adding artificial sweat to the cell culture medium and by moderately raising the temperature (to simulate the sweating conditions).

In all conditions, cell viability and cell metabolism were measured, comparing the same evaluations in an untreated negative control. Results were expressed as reduction of decrease of cell viability (%) and increase of cell metabolism (total protein dosage), calculated as difference in the experimental conditions with and without ÆCTive® treatment.

Results confirmed that ÆCTive® treatment has generally protected cell viability and improved cell metabolism during osmostress induction, activating cells in reacting to environmental stress. It could represent a good help for sensitive skins, whose dysbiosis is easily causes by stresses (sweat, swimming pool water, sea water …). In fact, the balancing activity against environmental changes, would be of help both for skin cells and for the microbiota living on the skin, that could be alter by the osmostress. The promotion of general homeostatic conditions against external and extreme stresses could lead to a general better status of skin microbiota, definitely driving to a healthier skin. Another *in-vitro* study has been performed on cells to evaluate the capability of the active ingredient to promote the synthesis of antimicrobial peptides, that contribute to the skin's antimicrobial defense when a growth in number of pathogens occurs.

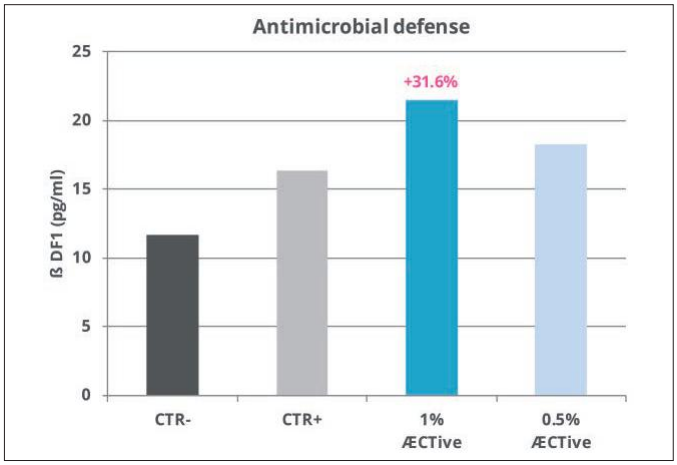


Picture 1: Cell viability evaluation



Picture 2: Cell metabolism evaluation

Human skin keratinocytes were treated with ÆCTive® at 3 concentrations (chosen after a preliminary cytotoxicity test) for 48 hours and with LPS (Lipopolysaccharides) for the subsequent 24 hours. In all the conditions β-Defensin 1 (β-DF1) expression was measured by ELISA assay. The same evaluations were performed on untreated negative control condition (CTR-) and positive control condition (cells treated with only LPS, CTR+). The results were expressed as β–DF1 (pg/ml) and as variation vs CTRs.



Picture 3: Antimicrobial defense on cell culture

The ingredient enhanced the surface expression of β-Defensin 1 peptides in cells treated with LPS, resulting in a protective effect, by balancing cells external environment. β-Defensin 1 expression is further increased respect to CTR+.

Starting from the *in-vitro* results, a step forward has been done *in-vivo,* where the active ingredient interacts with the complex system of the skin, made of human cells, tissues and structures together with microbiota microorganisms.

A double blind randomized study has been performed to evaluate the capacity of the ingredient in maintaining the balance of skin microbiota, even though skin was exposed to adverse environmental conditions. A clinical-instrumental study was carried out with a face cream placebo Vs cream with 1% ÆCTive® on 20 healthy Chinese subjects, aged between 18 and 80 years old, living in Beijing. This particular environmental conditions were characterized by high level of pollution that could negatively interact with the skin microbiota, leading to strong dysbiosis.

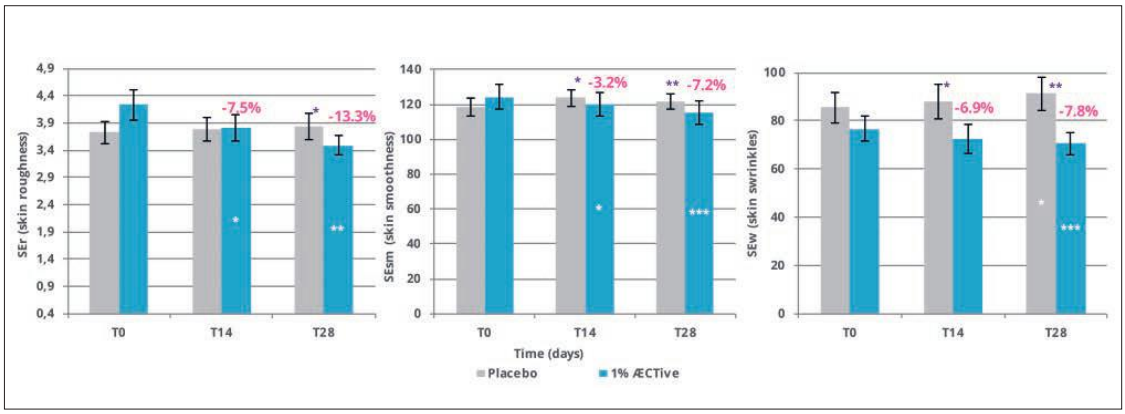
The women involved in the study applied the placebo cream in one side of the face, particularly on the cheek, and the active cream on the other side. By this way the inter-personal differences among microbiotas were reduced, trying to normalize the starting point of every person involved (placebo Vs active T0).

Product effects were evaluated after 14 (T14) and 28 (T28) days of daily product use by means of non-invasive bioengineering techniques, able to quantify the composition of skin flora (paying attention to bacteria) and by instrumental measurements to evaluate skin moisturization, elasticity and general profilometry.

The two different analysis (genomic on microbiome and instrumental on skin) were done to determine the effective microbiome equilibrium during time due to active ingredients use, followed by a better status of skin for hydration and elasticity. In fact, as the active ingredient is able to rebalance the microenvironment of the skin, there the microbiota is more balanced, leading to a better capacity of skin in doing its own activities.

After 28 days of daily application of products, the test shown that the cream containing ÆCTive® resulted in:

- a more balanced skin microbiome compared to placebo: *Actinobacteria* (the most present phylum on the skin) is a lot more similar to initial healthy status in the active group compared to placebo, and the same trend is in the genera analysis (data not shown)
- an improvement of skin moisturization respectively by +3.9% at T14 and by +5.5% at T28. This variation is statistically significant both compared to baseline and placebo formulation.
- an improvement of skin elasticity respectively by +3.6% at T14 and by +4.8% at T28. This variation is statistically significant compared to baseline and placebo formulation.
- a decrease of SEr parameter (related to a decrease of skin roughness) respectively by -7.5% at T14 and by -13.3% at T28. This variation is statistically significant compared to baseline and placebo formulation at the end of the study (T28).
- a decrease of SEw parameter (related to a decrease of skin wrinkles) respectively by -6.9% at T14 and by -7.8% at T28. This variation is statistically significant compared to baseline at T28 and compared to placebo formulation at each experimental monitored check.
- a decrease of R1 parameter (related to a decrease of skin roughness) respectively by -4.8% at T14 and by -6.5% at T28. This variation is statistically significant compared to baseline at T28.
- a decrease of R2 parameter (related to a decrease of maximum roughness) respectively by -2.7% at T14 and by -5.2% at T28. This variation is statistically significant compared to baseline at T28.
- a decrease of R5 parameter (related to a decrease of the arithmetical average roughness) respectively by -1.5% at T14 and by -9.7% at T28. This variation is statistically significant compared to baseline at T28.



Picture 4:
Skin profilometry
Legend:
* Significant (p<0,05)
** Very significant (p<0,01)
*** Extremely significant (p<0,001)

Conclusions

Recent studies have shown that microbe populations that live inside, and on the human body account for almost 40 trillion of bacterial cells. Almost all these bacteria have a positive action on the host organism. Microorganisms are fundamental for the production of metabolites that are functional to our health. They give protection to the skin and inhibit pathogens proliferation both inside and outside the body. They play also an essential role in the modulation of our immunogenic system. For all these reasons, it is of paramount importance the regulation of the body microbiomes through specific ingredients for development of optimal beauty-cosmetics and the prevention and treatment of several diseases.

The present work provides evidence of skin benefits after topical applications of cosmetic formulas containing ÆCTive®. The ingredient, developed through cutting-edge biofermentation technology, is a novel innovation for cosmetic formulators seeking to effectively protect each person's unique and varied microbiota.

The ingredient is an osmolyte able to maintain the environment around cells in equilibrium against external stresses: activity demonstrated by *in-vitro* tests.

This ingredient is able to balance the existing bacterial environment on the skin, so the ecosystem microbiota: activity demonstrated by *in-vivo* genomic tests, showing a better equilibrium of microbial species after active treatment. As demonstrated by in-vivo dermatological tests, if the skin microbiota is in homeostasis, the skin can work better.

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After a high school graduation in languages, she got her degree in biology at the University of Milan, in 2013. She has focused her lab experience on skin histology and molecular biology, with a master thesis on wound healing process performed at the National Centre for Biological Sciences in Bangalore (India). In 2014 she started her experience in the R&D of Principium, a small company which became part of ROELMI HPC in 2017.

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