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Breaking New Ground in the Exploration on Skin Microbiome

Biotechnological Active Ingredient, Protecting Skin Microbiota

Biotechnological Active Ingredient, Protecting Skin Microbiota

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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 easily disrupted by stresses. An alteration of skin microbiota may leads to dysbiosis, which has been associated with skin disorders. The present paper aims at demonstrating the efficacy of a specific selected bacterial strain from cutting-edge biofermentation technology able to maintain skin homeostasis and consequent prevention of skin disorders.

Introduction

This little but important World is essential for the skin to be a complete barrier, accomplishing its activities 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 also intra-personally during our lives, link to age, change of lifestyle or to the external stresses we are submitted to [4]. Different body sites can have completely different skin microbiota configurations, both inter- and intra-personally, linked to 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 moist, dry and sebaceous skin sites [5].
Despite continuous changes in its composition, when the body is in a homeostatic status, skin microbiota is an equilibrium between protective and pathogens microorganisms, living together in a complex community and having 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. Anyway, going into more specific taxonomic classification, as class or genus or species, we can find differences among peoples' microbiomes even though looking at subjects with very similar age, lifestyle, and from the same ethnicities.
The general truth for everyone seems to be really the homeostatic condition of skin microbiota with its singular peculiarities that allows a healthy status of the skin.
However, everyone's microbiota balance can be easily disrupted by external and internal stresses. A strong change in the environment, for example, can alter skin microbiota leading to its dysbiosis, which has been often associated with skin disorders (skin inflammation, sunburn, dandruff, atopic dermatitis, psoriasis, acne). To avoid microbiota dysbiosis, i.e. maintaining microbiota equilibrium, may prevent skin disorders.
Moreover, it is essential to maintain skin microbiota's natural biodiversity in different body sites, in order to let the skin barrier work 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 good performances with the lowest impact on skin homeostasis. The objective of this study is to develop a cosmetic ingredient able to maintain the equilibrium of the microbiota with consequent prevention of skin disorders.

Methodology

ROELMI HPC has been working for more than 10 years on bio-fermentation technology, focusing on production of different proprietary probiotic strains and pure postbiotics.
About probiotics, every investigated strain 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.
Then, a step forward made by realizing probiotics for cosmetic applications. A line of inactivated probiotics has been standardized thanks to heat technology after bacterial fermen-

tation. By means of this approach, probiotics are no longer living cells, and can be used as cosmetic ingredients. In fact, they still maintain their mimetic effect on the skin immune system, since they do not lose their cell shape once inactivated. It means that the body is able to recognize them as bacteria, as cells are not destroyed like in lysate ingredients, so skin natural defense are reactivated.
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 development on the activity of postbiotics produced by fermentation, with a targeted effect on microbiota equilibrium. The last one is a powerful pure molecule able to rebalance the skin microenvironment against external stressors demonstrating a peculiar protection activity of microbiota equilibrium and biodiversity. This ingredient, marketed under the trade name of ÆCTive® (INCI: Ectoin), acts as a promoter of healthier skin by regulating skin homeostasis. Skin moisturization, elasticity and the general skin profilometry are 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 the company's 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. The company 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, the company 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.
The company, 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. 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, the company 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 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.
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 cells 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.
A production and applicative patent registration is ongoing on 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 to the cell culture medium:

- Salt (to simulate marine environment)
- Chlorine (to simulate swimming pool environment)
- Artificial sweat and by moderately raising the temperature (to simulate the sweating conditions).

In all conditions, cell viability (**Fig. 1**) and cell metabolism (**Fig. 2**) 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 Ectoin 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, seawater…). 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 to promote the synthesis of antimicrobial peptides that contribute to the skin's antimicrobial defense when a growth in number of pathogens occurs.
Human skin keratinocytes were treated with Ectoin 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+).

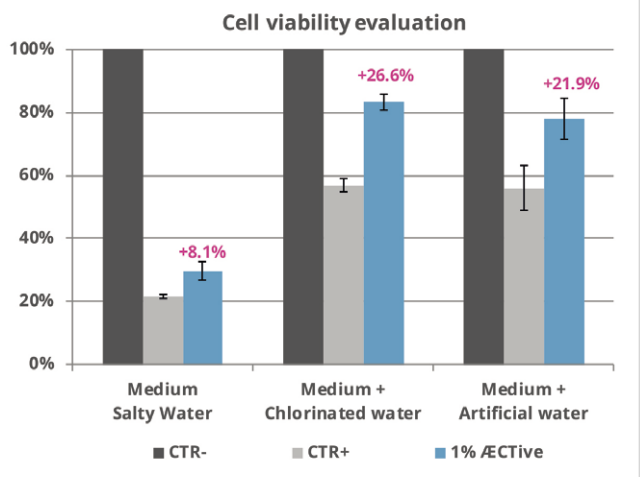

Fig. 1 Cell viability evaluation.

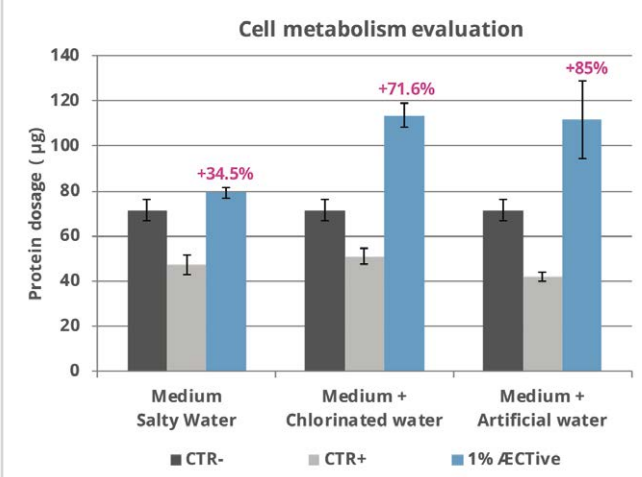

Fig. 2 Cell metabolism evaluation.

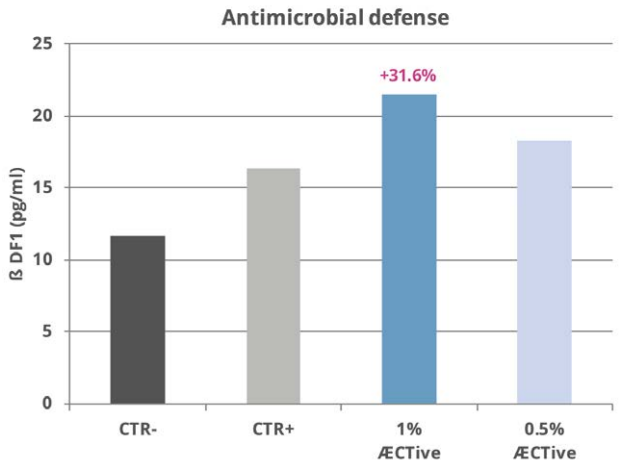

Fig. 3 Antimicrobial defense on cell culture.

The results were expressed as β–DF1 (pg/ml) and as variation vs CTRs. (**Fig. 3**)

The ingredient enhanced the surface expression of ß-Defensin 1 peptides in cells treated with LPS, resulting in a protective effect, by balancing cells external environment. ß-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% Ectoin 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 and 28 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 Ectoin resulted in (**Fig. 4**):

- 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.

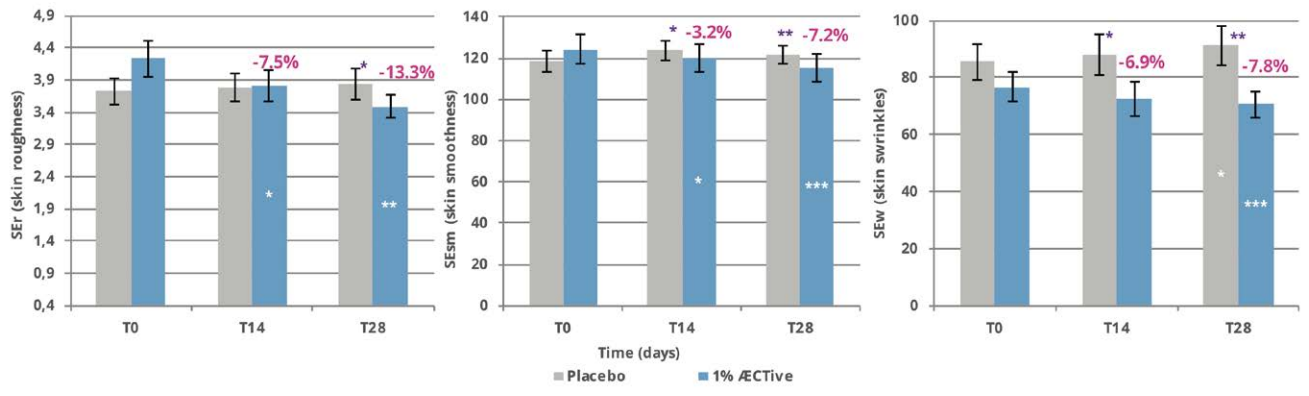

Fig. 4 Skin profilometry (Legend: * Significant (p<0,05); ** Very significant (p<0,01); *** Extremely significant (p<0,001)).

- 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.

Further Investigations: Improving Skin Appearance on Adults Affedted by Active Acne

Ongoing study to assess the efficacy of a complete treatment (food supplement + cosmetic product) in improving skin appearance on adult subjects affected by active acne. Controlled study vs placebo and reference product.
A board-certified dermatologist according to the ethical principles for medical research carries out the study, enrolling 80 subjects. In particular, female subjects aged between 18 and 50 years old showing acne severity from 1 to 3 according to IGA scale.
The study foresees 2 months of products use. Checks are fixed before product use (T0) and after 28 (T28) and 56 (T56) days of products use. According to a predisposed randomization list included subjects will be divided in 4 groups:

- G1: 20 subjects - complete active treatment (cosmetic product + food supplement)
- G2: 20 subjects - active cosmetic product + food supplement placebo
- G3: 20 subjects - cosmetic reference product + food supplement
- G4: 20 subjects – cosmetic reference product + food supplement placebo

Measured parameters:

Iinstrumental Evaluation

- Skin sebum by Sebumeter, measuring the content of sebum on the skin.
- Skin moisturizing. The measurement of the skin moisture is based on the internationally recognized CORNEOMETER® method (Courage+Khazaka, electronic GmbH).
- Skin pH evaluation. The used instrument is the SKIN pH-METER 905®, Courage + Khazaka GmbH.

Clinical Evaluation by means of dermatologist analysis:

- The numbers of acneic lesions (papules, pustoles, open and closed comedones)
- Clinical evaluation of skin complexion evenness
- The improvement of the skin inflammatory status of the area interested by acneic lesions

At the end of the study (T56) subjects are asked to reply to a self-assessment questionnaire.

Conclusions

Recent studies on skin microbiota are opening a series of future developments in personal care field. Cosmetics are no longer acting on human epidermis, but companies today are considering the effect of products on skin microbiota, which varies a lot among people. Cosmetics should treat this microorganism's community by protecting its natural stability and biodiversity, without altering its composition.
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 the innovation for cosmetic formulators to effectively protect each person's unique and varied microbiota.

The active product is exclusive distributed in Germany by S.GOLDMANN GMBH & CO.

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The corresponding author has a working relationship with ROELMI HPC srl which is developing products related to the research being reported. Commercial articles about the topic have been submitted to magazine for publications: Eurocosmetics (6-2019), Personal Care Magazine (September Asia issue

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