

# Biotechnological approach to mitigate skin exposome

## A specific guardian for microbiota care

MARIA GIOVANNA BRUNO\*, STEFANIA ZANZOTTERA

\*Corresponding author

ROELMI HPC Srl, Origgio, Varese, Italy

### Abstract

Skin is the perfect ecosystem in which billions of microorganisms live and continuously interact with skin cells providing a safe environment capable of maintaining biodiversity. However, chronic exposure to UV rays, pollution and other substances negatively affects our skin microbiota. Preserving the diversity of skin microorganisms plays an important role in defending the organism against the activation of immune phenomena that trigger inflammation. The alteration of skin microbiota, known as dysbiosis, has been associated to exposome-induced damages manifesting as skin disorders and, it concurs to weak skin barrier integrity and consequent sensitivity.

This work aims at presenting the evidence of effectiveness towards a biotechnological-based dermobioc approach for the prevention versus skin exposome undesired distresses and for the rebalance of skin environment.

### Keywords:

- Microbiota care
- Skin-environment care
- Ectoin
- Skin distress
- Microbiome

### INTRODUCTION

Skin microbiota represents all the microorganisms living on the upper layers of the skin, the interaction between commensal microorganisms and skin cells is responsible of balanced biodiversity, an essential condition called eubiosis.

When life begins, skin microbiota starts to form and balance in terms of composition and like a fingerprint represents a unique feature of every single person chaperoning throughout the entire life cycle (1).

Microorganisms are essential to maintain skin homeostasis; for that reason skin microbiota is involved in defense against pathogens, external stresses that can occur by altering the skin ecosystem; these alterations are shown as a changing in microorganism number and also in taxonomical composition (2).

When environmental stresses occur, the skin ecosystem equilibrium is lost and natural immune defenses are activated to counteract the inflammation occurring (3). Several ingredients have been developed aiming at serving the microbiota care. An ingredient targeting the rebalance of skin ecosystem is represented by the subject of this study: ectoin. In fact, the protective properties of ectoin, aimed at ensuring the survival of extremophilic microorganisms, could be transferred to human skin microbiota designed formulas. The ingredient is a pure molecule naturally found in extremophilic microorganisms living in strong environmental conditions and firstly isolated by Galinski and colleagues from the bacterium *Ectothiorhodospira halochloris* found in Wadi Natrun (Egypt). Ectoin is an amino acid derivative that acts as an osmolyte compound, whose main function is to balance the extracellular environment salt concentration. Its bacteria production concurs to equilibrate the surrounding environment and protect these biopolymers such as cytoplasmic enzymes, against damage caused by reduced water activity and against external stresses (i.e. strong UV rays, chemical agents...) (4).

### MATERIALS AND METHODS

Starting from a 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.

ÆCTive® (INCI: *Ectoin*) is a pure molecule, from a non-GMO bacterial strain, showing peculiar activity against strong environmental stresses. ROELMI HPC has transformed this extraordinary discovery for extremophilic bacteria into a unique product for cosmetic applications demonstrating through specific metagenomic tests that the ingredient is able to keep the skin microbiota in balance, ensuring the well-being of the skin. *In vivo* tests show the efficacy of the product to help skin microorganisms to survive, proliferate and protect the skin by helping cells to recover from acute stress.

Safety and efficacy tests took place at Complife Italia Srl and Asia, an independent testing laboratory for safety and efficacy assessment of cosmetics, food supplements and medical devices.

### Assessment of safety parameters

Safety tests have been performed according to the legislation in force. *In vitro* MTT assay for the evaluation of the cytotoxic potential. *In vitro* Het Cam Test for the eye irritating potential cytotoxic potential (5 % in corn oil). Repeated patch test for skin sensitization potency (occlusive method on 50 volunteers as is). *In vitro* test method for the prediction of the irritation of oral mucosa.

### Assessment of the capability to contribute to the maintenance of cell homeostasis

The capability of the cosmetic active to contribute to the maintenance of cell homeostasis (measured by means cell viability and cell metabolism) after osmotic stress 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 osmotic stress conditions have 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 (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 (1% active ingredient) and without the active treatment (Control +).

### Assessment of the capability to modulate antimicrobial defense on cell culture

Another in-vitro study has been performed on cells to evaluate the capability of the active ingredient to promote the synthesis of antimicrobial peptides, which contribute to the skin's antimicrobial defense when a growth in number of pathogens occurs.

Human skin keratinocytes were treated with tested item 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  $\beta$ -Defensin 1 ( $\beta$ -DF1) expression was measured by ELISA assay. The same evaluations were performed on untreated negative control condition (Control-) and positive control condition (cells treated with only LPS, Control+).

The results were expressed as  $\beta$ -DF1 (pg/ml) and as variation vs Controls.

### Assessment of the efficacy in boosting the activity of Sodium Hyaluronate (Medium Molecular Weight)

Instrumental study is carried out on 20 female subjects aged between 30 and 65 years with normal to mixed skin. All the volunteers applied on the right side of the face the placebo cream containing medium molecular weight sodium hyaluronate and on the left side of the face they applied the active cream consisting of the basic formula plus medium molecular weight sodium hyaluronate and tested item.

The study duration is 28 days. Product efficacy is assessed by means of non-invasive techniques which allow to quantify skin moisturization, skin sebum content and skin pH after 7 (T7), 14 (T14) and 28 (T28) days of product use.

### Assessment of the efficacy to maintaining the balance of the skin microbiome

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 extreme environmental conditions. Particularly, the study checked the composition of skin flora before and after treatment, paying attention to pathogens species reduction and resident species balancing.

Moreover, the study was held in particular environmental conditions as high level of pollution (Beijing) that could negatively interact with the skin microbiota, leading to unbalanced conditions.

The clinical study was carried out with a face cream placebo Vs cream with 1% tested item on 20 healthy Chinese subjects, aged between 18 and 80 years old, living in Beijing.

The women involved applied the cream with 1% of *Ectoin* in half side of the face and the placebo cream in the other half side.

Product effects were evaluated after 14 (T14) and 28 (T28) days of daily product application by means of non-invasive bioengineering techniques, able to quantify the composition of skin flora (paying attention to pathogens) and by instrumental measurements to evaluate skin moisturization (based on CORNEOMETER®), elasticity (based on Cutometer® MPA 580, Courage+KHzaka, electronic GmbH) and general profilometry (based on VisioScan VC98 USB, Courage+KHzaka, electronic GmbH).

## RESULTS

As reported in Figure 1 and Figure 2, results confirmed that the active treatment demonstrates beneficially actions in protecting cell viability (+8.1%, +26.6% and +21.9 in different experimental conditions) and improving cell metabolism (+34.5%, +71.6% and +85% in different experimental conditions) during osmotic stress induction, by activating cells in reacting in a positive pathway to environmental stress balancing the ideal water content from the inside and outside of cell membranes.

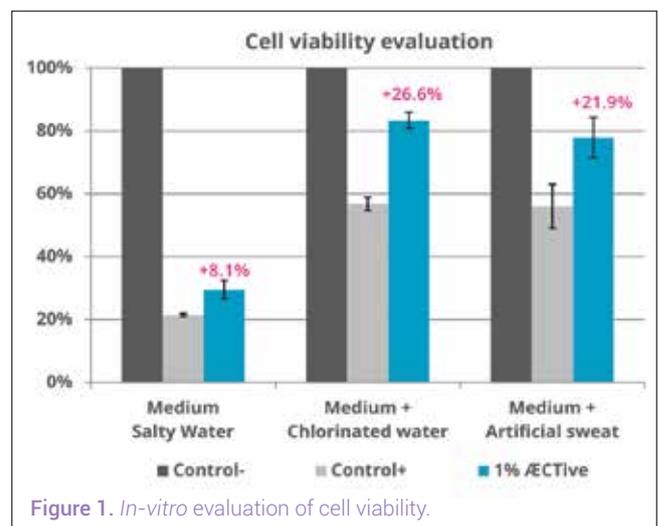


Figure 1. In-vitro evaluation of cell viability.

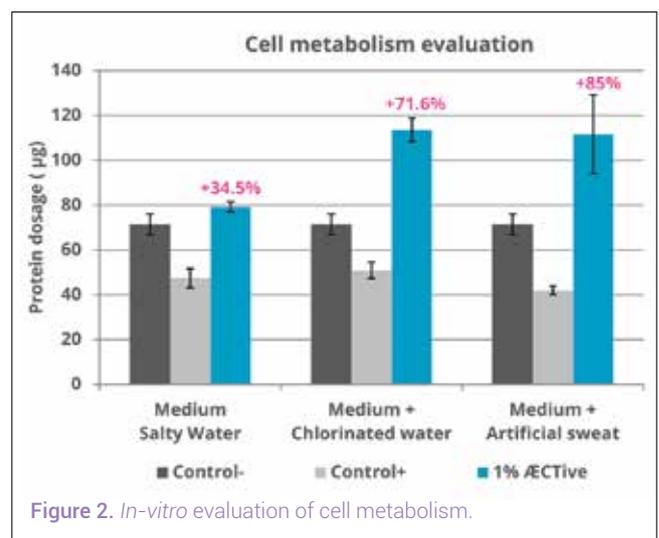
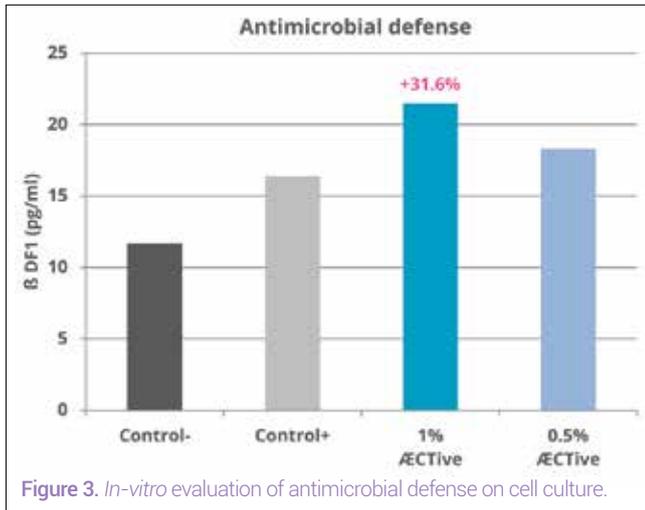


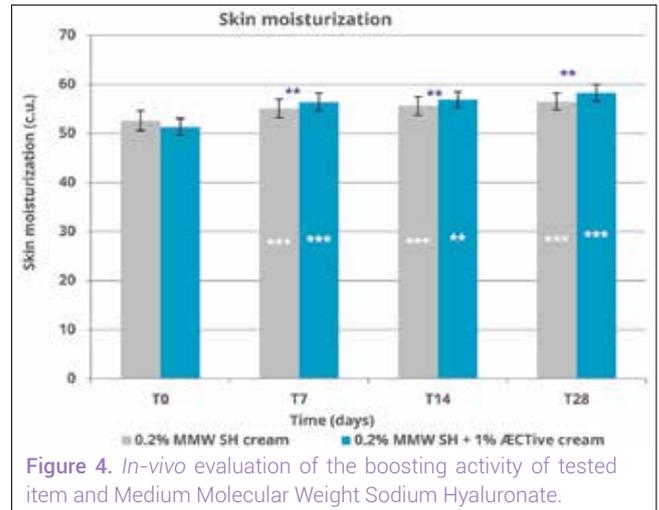
Figure 2. In-vitro evaluation of cell metabolism.

The ingredient enhanced the surface expression of  $\beta$ -Defensin 1 peptides in cells treated with LPS, resulting in a protective effect, by balancing cells external environment.  $\beta$ -Defensin 1 expression is further increased respect to Control+. The  $\beta$  Defensin expression is further increased with 1% tested item respect to Control+.



Tested item demonstrates a capacity in boosting the moisturizing effect of medium molecular weight Sodium Hyaluronate (MMW SH). A statistically significant increase of skin moisturization was observed in both groups. Differences between the two groups are statically significant starting from 7 days of products' use. This variation indicates that the tested item is effective in boosting the moisturizing effect of sodium hyaluronate. The ingredient performed great ability in increasing skin moisturization (+14.3% vs T0 at the end of treatment) and increasing sebum content (the skin sebum content is not indicative of oily skin or skin tendency to be oily) and an unaltered skin pH (pH value around 5,3-5,5) data not shown. Figure 4 reports the mean data obtained at each experimental check for the analyzed skin parameter. Data are reported as mean  $\pm$  SEM. \*\*\* p<0.001 vs T0, \*\* p<0.01 vs T0, \*\* p<0.01 vs other item.

After 28 days of daily application of products, the metagenomics test expressed by Phyla, Class, Order and Genus shown that the cream containing the tested item 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
  - a microbiota composition in samples collected from the side of the face treated with Base cream containing tested item shows less variation than the samples collected from their face counterparts and treated with placebo.
- a reduction of 45,2 % (compared to T0) of *Corynebacterium 1* related with skin sensitization and skin inflammation. It is demonstrated that after 28 days of daily use, the cosmetic product containing tested item helps the normal balance of skin microbiota. The two different analysis (genomic on microbiome and instrumental on skin) determine the effective microbiome equilibrium during time followed by a better status of skin hydration and elasticity.



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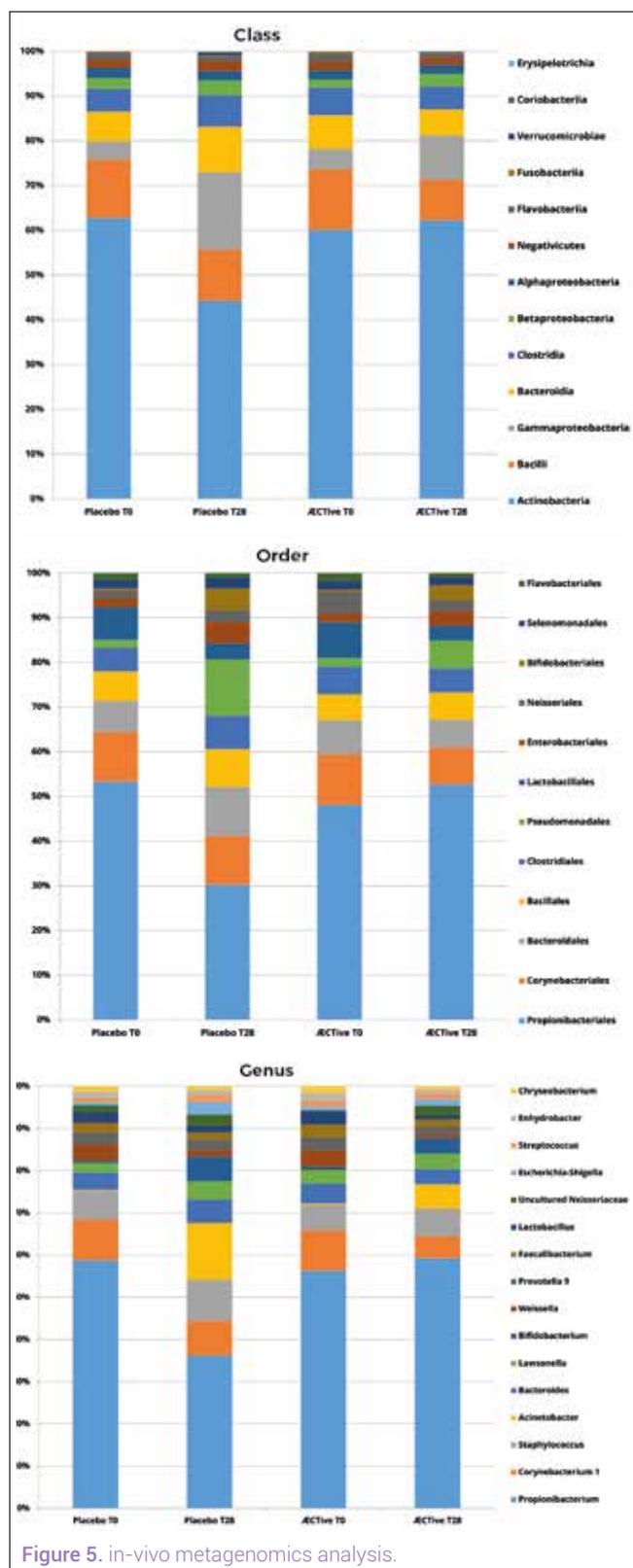


Figure 5. in-vivo metagenomics analysis.

## DISCUSSION

Recent studies have shown that human body microbe populations count up 40 trillions 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 immune responses.

For all these reasons, it is of paramount importance the regulation of body microbiomes ecosystem through specific ingredients for the prevention and treatment of several diseases that could lead to skin exposome phenomena. Focusing on the results shown above, tested item, included in different formulations, demonstrated to have beneficial effect in restoring balanced condition of skin microbiota environment.

Tested item could represent a good help for sensitive skins, whose dysbiosis is easily caused by exposome-related stresses (chronic exposure to UV rays, pollution and other external aggressors). 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 altered by different osmolestress. Extreme environmental stresses such as, frequent washes, dry winter weather, less gentle surfactants, may cause skin dryness damaging skin barrier and accelerating skin aging. The promotion of ideal equilibrium conditions against external and extreme stresses could lead to a healthier status of skin microbiota, driving to skin well-being.

## CONCLUSIONS

The work provides evidence of skin benefits after topical applications of cosmetic formulas containing an osmolyte acting as guardian for skin microbiota. The ingredient, developed through cutting-edge biofermentation technology, is a novel innovation for cosmetic formulators seeking to effectively protect individual and varied microbiota.

This evidence represents the starting point for driving further investigations in skin microbiota care topic, characterized by growing interest from both industry researchers and end-customers.

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## ABOUT THE AUTHOR

**Maria Giovanna Bruno** majoring in Chemistry and Pharmaceutical Technology at University of Calabria. She obtained 2 master's degrees in Management of the Cosmetic industry at Sapienza

University (Rome) and in Science and Cosmetic Technology at University of Pavia.

After an experience as pharmacist and cosmetic formulator, she joined ROELMI HPC in 2020 as Product Manager – Personal Care actives.