Probiotic-derived ingredient for skin microbiota care assessment of the efficacy in well-being cosmetics

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PEER REVIEWED

Abstract

The beneficial effects of probiotics on human health are widely recognized. Recent findings demonstrate the excellent opportunity also for topical applications, highlighted by the growing demand from worldwide end-users.

This work aims at presenting the evidence of effectiveness towards a new cosmetic ingredient, based on *Lactobacillus rhamnosus* LRH020. Specially designed for promoting the skin ecosystem's well-being, the result is the novel ability to strengthen the skin barrier and its natural defenses by rebalancing skin microbiota. In addition, the study will describe a series of *in-vitro* and *in-vivo* tests supporting the scientific efficacy background of the ingredient.

Keywords

- Microbiota Care
- Probiotics
- Cosmetics
- *Lactobacillus rhamnosus* LRH020
- Healthy Ageing
- Peer Reviewed

INTRODUCTION

The cosmetic market is experiencing an incredible revolution regarding microorganisms, considering both a target and potential topical applications. More and more products containing pre-, pro- and post-biotics have been launched with claims referring to the rebalance of skin's microbiota. Along with the products growing quantity, confusion about the ingredients used for the treatment of the microbiota and their classification also grows. The safest way to insert microorganisms as real ingredients is to make them inactivated and more stable within a cosmetic formula.

Probiotic-derived is currently classified as 'postbiotic' and maintains structural integrity by preserving cells membrane. This process allows to have an interaction comparable to probiotics and to guarantees some benefits associated with live microorganisms such as strengthening skin barrier and its natural defenses (1).

MATERIALS AND METHODS

Starting from its deep knowledge of probiotics development for nutraceutical applications, ROELMI HPC has moved to the cosmetic market, designing a specific ingredient targeting the well-being of the skin ecosystem (2).

EquiBiotics™ LRh (INCI: *Maltodextrin, Lactobacillus Ferment*) is a probiotic-derived ingredient obtained from the proprietary strain *L. rhamnosus* LRH020, selected for its strain-specific activity focused on preventing bacterial colonization and modulating the immune response. The ingredient represents a valid approach to beauty routine, targeting skin microbiota comfort. The key is to promote a balanced ecosystem while preserving or restoring the natural harmony between the skin barrier and its microbiota (3). In its activity, the ingredient performs a triple mechanism of action by:

1. Adopting a molecular biomimetism: potential recognition thanks to the preservation of bacteria integral structure by pursuing cross-talks specific pathways to alive probiotics
2. Preventing pathogens colonization: by counteracting pathogens invasion through:
   a) Exclusion: physical occupation of adhesion sites (4)
   b) Displacement: pathogen removal from adhesion sites
   c) Competition: quicker settlement than pathogens in adhesion sites (5)
   d) Exerting a "prebiotic" effect and modulating pathogen growth: *probiotic-derived ingredients* could be a metabolic substrate for other microorganisms by rebalancing the resident microbiota community by promotiing of commensal bacteria and avoiding pathogens aggressions (6, 7).

The ingredient is safe for cosmetic use as per the evaluation performed by an independent testing laboratory for safety and efficacy assessment of cosmetics, food supplements and medical devices. The ingredient resulted in non irritant according to the in vitro R.B.C. Test (eye irritating potential) (8) and H.I.R.P.T. (skin sensitization potency).

Assessment of the capability to modulate inflammatory status

The capability of *inactivated lactobacilli* to contribute in the modulation of inflammatory status has been performed on cell cultures (human keratinocytes), in presence of an inflammation agent (Sodium Dodecyl Sulfate - SDS 0.05 mg/ml) (9, 10). The anti-inflammatory activity was expressed by TNF-alpha dosage in the culture medium (11). Furthermore, the same evaluations were performed on untreated negative control conditions (CTR-) and positive control conditions (cells treated with only SDS, CTR+) and toward live probiotic microorganisms after inflammation induction (12).

Assessment of the capability in preventing pathogens colonization

Another *in-vitro* study has been performed on cells to evaluate the bacteriostatic activity against *Cutibacterium acnes* (ATCC 11827), a gram+ commensal bacteria, identified as an etiological agent responsible for cutaneous distresses, like acne (13). Parameters are expressed in log CFU/ml and were evaluated at T0, after 8 hours (T8) and 24 hours (T24). Positive control (CTR+) shows the culture medium with the addition of $10^{\wedge 5}$ CFU/ml of *C. acnes* as a title. Furthermore, the tested item has been subjected to analysis to assess its capability

to form a co-aggregate with *Escherichia coli* and *Candida albicans* (14). Pathogens and inactivated microorganisms have been mixed 1:10 in a buffer solution to evaluate the visual precipitate formation. The time of flocculation was recorded and precipitates were fixed for SEM analysis.

Assessment of "prebiotic" effect

In-vitro study carried out on probiotic cultures added in several petri-dish at 0.16 mg/mL of Glucose and tested item. After 24h at 37°C, growth has been measured by count.

Assessment of the efficacy on SLS-induced skin redness and on skin barrier

The daily application of toiletries that may contain aggressive chemical agents can lead to skin sensitization. A placebo-controlled study enrolling 20 healthy female subjects was performed (15). Skin redness (a* parameter) was evaluated 24 hours after a SLS patch application (T0+24H), after the measurement placebo and active creams started to be used daily by volunteers for 14 days (T14). After the treatments, skin redness was evaluated after 24H of SLS patch application (T14+24H), and compared to placebo. Furthermore, product efficacy in improving skin barrier conditions (in terms of decrease of Trans Epidermal Water Loss) was evaluated before and after 14 days of product use on intact skin (not SLS-treated).

Assessment of the efficacy in reducing UV-induced skin redness and skin aging signs

UV damage is one of the main causes linked to uneven skin complexion and premature aging. A placebo-controlled study was carried out on 20 female subjects with skin phototype I, II or III (according to Fitzpatrick classification) and clinically showing mild to moderate skin aging signs, average age 55 years old.

Product efficacy on skin redness was assessed by means of a short-term test through the instrumental evaluation of skin redness (a* parameter). The evaluation was performed before (T-1), after the induction of damage (T0) and then 1 and 2 hours (T1h, T2h,) after products application in the four treated (2 areas for each product) and two untreated areas. The UV-induced redness of the skin was evaluated by means of a spectrophotometer/ colorimeter CM-700D (Konica Minolta). Product efficacy on skin aging sings was assessed by means of a long-term test on skin moisturization, skin elasticity (R2 parameter), skin firmness (R0 parameter) and wrinkle depth after 14 and 28 days of products use Material: Corneometer® CM 825 (Courage+Khazaka, electronic GmbH).

RESULTS

As reported in Figure 1, a strong and significant reduction of the TNF-alpha release was measured both in the cell cultures treated with alive microorganism and *inactivated lactobacilli* (* p<0.05 vs CTR-, ° p<0.05 vs CTR+). In comparison to alive probiotic strains, the tested item comparably modulates the release of TNF-α. This result is linked to its structural integrity, preserved during the technological process, allowing modulating specific inflammation thanks to a molecular biomimetism mechanism of action performed.

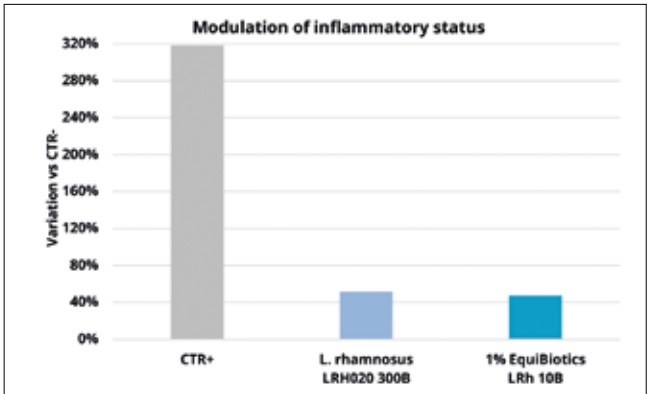

Figure 1. Modulation of inflammatory status.

At different dosages (1% and 2%), the ingredient can also induce a bacteriostatic activity by counteracting the pathogen growth (Figure 2). This is linked to the ability of the active ingredient to form a co-aggregate behavior with other microorganisms, for example *Escherichia coli* and *Candida albicans* (data not shown).

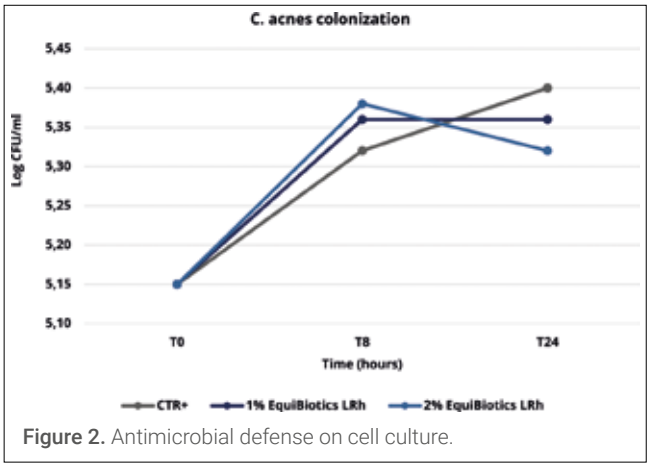

Figure 2. Antimicrobial defense on cell culture.

Another interesting finding is related to the capacity to exert a "prebiotic" effect on probiotic strains, promoting growth as efficiently as glucose (Figure 3).

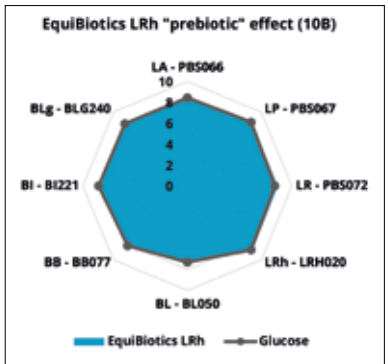

Figure 3. "prebiotic" effect.

The protection activity performed by the tested item was confirmed by the statistically significant and influential results in decreasing the SLS-induced skin redness by -17.0% (Figure 4). Furthermore, the active ingredient improves skin barrier function by preventing the water loss content (16). In fact, after 14 days of treatment, 1% of active ingredient shows a statistically significant decrease of TEWL by -12.6% (data not shown).

The ingredient has been demonstrated to be also efficient in downregulating skin redness after UV exposure. 1% *inactivated lactobacilli* demonstrated a decrease of skin redness linked

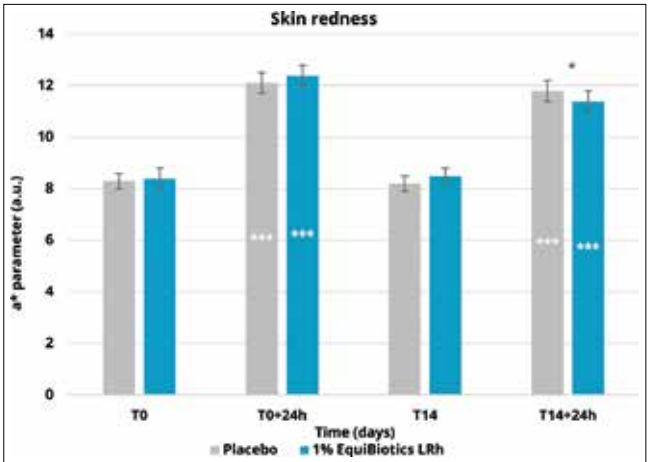

Figure 4. Reduction of the SLS-induced redness.

to UV exposure up to 11% after 2 hours of active cream application (data not shown).

Concerning pro-age effects, results reported in Figure 5 demonstrated:

- An increase of skin moisturization up to 7.9% and 13.9% after 14 and 28 days respectively.
- An increase of skin elasticity up to 2.7% and 6.0% after 14 and 28 days respectively.
- A decrease of R0 parameter (related to a rise of skin firmness) by -1.9% and -5.8% after 14 and 28 days respectively.
- A decrease of wrinkle depth parameter respectively by 12.4% at T14 and by 15.8% at T28.

DISCUSSION

The innovative approach, which aims to treat the skin no longer as a static barrier but as a unique ecosystem, opens to ever-higher demand of formulators to include in cosmetics formulas a type of ingredients that can positively interact with microorganism's population living on and in the skin layer.

For this reason, the study and development of a new class of specially designed active ingredients are of fundamental importance.

Focusing on the results reported above, the tested item has been shown to have beneficial effects on skin barrier, understood as a complex and ever-changing ecosystem in continuous evolution, through a mechanism of daily cross-talk between skin's microbiota and cells.

In particular, the ingredient demonstrates high performing efficacy towards multifactorial aggressive agents such as UV rays, chemical agents also helping avoid pathogens colonization. Thanks to their "prebiotic" effect, inactivated *Lactobacillus rhamnosus* LRH020 become nourishment and support for resident microbiota growth.

The result is the novel ability to strengthen skin barrier and its natural defenses promoting skin ecosystem well-being (2). Those specific benefits that traditional cosmetics can verify, such as moisturization, skin elasticity, and wrinkle reduction, are recorded as secondary benefits obtained because of skin ecosystem well-being.

CONCLUSIONS

The work provides preliminary evidence of the beneficial effects linked to the topical applications of *inactivated lactobacilli.*

The ingredient, developed through cutting-edge technology that preserves the integral bacterial structure, could therefore be considered a step forward in researching new ingredients capable of interacting with the skin microbiota. Last but not least, we need to keep in mind a very important aspect that has recently become almost fundamental in cosmetic formulations: the sensorial profile.

Thanks to a deep investigation on the topic, ROELMI HPC has identified and developed a useful method and a valid solution to introduce complex structures, such as *lactobacilli*, in the cosmetic formula while preserving sensoriality.

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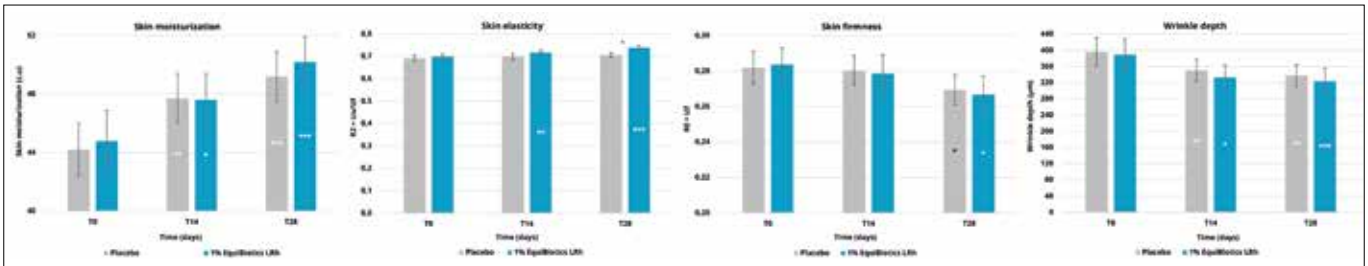

Figure 5. Skin profilometry
Legend: * Significant (p<0,05), ** Very significant (p<0,01), *** Extremely significant (p<0,001).

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