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Probiolift™

Probiotic for graceful aging

by Beauty Creations
another Care Creations™ product group Inspired by Life

Global Except US

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SUMMARY FILE

Probiolift™	BC10157
Origin - Description	Dormant, living cells of *Lactobacillus crispatus*, a beneficial microorganism naturally occurring on young skin, for graceful aging
Regulatory data	
INCI	Maltodextrin (and) Lactobacillus
China	All components are listed in Inventory of Existing Cosmetic Ingredient in China (IECIC 2021)
CAS#	9050-36-6; 99999-99-6
Appearance	White to beige powder
Preservative	None
Natural labels	None
Naturalness content (ISO 16128)	100% from natural origin
Cosmetic use	
Properties	Helps to activate the synthesis of collagen I and collagen V Helps to decrease the frequency of cell contraction Contributes to protect the skin cells against oxidative stress Reduction of wrinkles appearance and improvement of the dermis density
Applications	Anti-aging and photoaging face care
Formulation data	
Concentration of use	0.05%
Solubility	Dispersible in oils and fat
Incorporation method	Probiolift can be dispersed into cosmetic product at room temperature for cold processing or below 70°C
Optimal pH	4-6

BIOTIC INGREDIENT FOR HEALTHY AGING

Biotic ingredient for healthy aging

The anti-aging cosmetic market is thriving, driven by emerging consumer demands and new technologies. At the same time, consumer antiaging demand has shifted toward functionality, healthy aging and natural safe ingredients.

We name biotic ingredients, ingredients issued from specific microorganisms that are beneficial for skin beauty. Probiolift is one of the two newly developed biotic ingredients (Probiolift and Postbiolift) to support healthy and graceful skin aging. Unlike other biotics existing on the market, the newly developed actives are the first to use a bacterium naturally present on the skin: *Lactobacillus crispatus (L. crispatus)*, a bacterium which has been found to decrease with age.

Anti-aging evolving need

Skin aging is a complex biological process influenced by a combination of endogenous or intrinsic and exogenous or extrinsic factors. Because skin health and beauty are considered one of the principal factors representing overall "well-being" and the perception of "health", several anti-aging strategies have been developed during the last years (Ganceviciene *et al*, 2012).

Anti-aging need is evolving. Healthy aging is a major beauty trend helping people across demographics live longer and maintain overall health (Mintel – Cater to the evolving needs of healthy aging consumers/ industry trend). Middle ager (above 50 years old) wants to live positive aging. 47% of US consumers aged 55-64 say that being healthy give them sense of pride. They are suffering from instability and stress and are looking for healthy ingredients like probiotics.

Probiotic, postbiotics and cosmetics

There is currently some confusion with the use of the word probiotic. In the cosmetic industry, the use of the word probiotic is currently often extended to products derived from yeast and bacteria (extracts, filtrates, lysates, not live microorganisms), which corresponds better to the definition of postbiotics. True probiotics are live (active or dormant) microorganisms added to a cosmetic product to deliver cosmetic benefit to the host when used in adequate amounts.

Probiotics are considered as healthy and natural ingredients by consumers. They have been used for decades as oral supplement to boost immunity and treat some gastrointestinal diseases (Stavropoulou and Bezirtzoglou, 2020). Recently several publications (Habeebuddin *et al*, 2022; Loulou and Panayiotidis, 2019) mentioned their potential to treat skin diseases and conditions even if applied topically.

The probiotic cosmetic product market including also postbiotic and other probiotic derivatives is forecasted to grow at a CAGR of 7.2% during the forecast period: 2021-2027 (Mordor Intelligence, 2021). This trend is observed in many regions (Europe, Asia and USA). New product lines are launched in this market, but mainly with postbiotics, contributing to market growth (Puebla-Barragan *et al*. 2021). True probiotics (live microrganisms) are still an emerging field with a very small number of products available targeting skin conditions such as acne or atopy (Fourniere *et al*, 2020; Lebeer *et al* ,2022).

Probiotics defined as "Live microorganisms that when administered in adequate amounts confer health benefit to the host" are becoming increasingly popular and marketable. In recent years the number of product termed "probiotic" has increased on cosmetic market, however most of them are not living microorganisms. For esthetic anti-aging application, data are scarce, with only 2 examples of such benefits reported. Dietary supplementation with probiotic *Lactobacillus plantarum* HY7714 isolated from breast milk of healthy women was reported to reduce facial wrinkle depth (Lee *et al*, 2015) and *Nitrosomonas eutropha*, an ammonia oxidizing bacteria isolated from soil, has been shown to tend to decrease wrinkle appearance when applied as a mist on the skin (Notay *et al,* 2020).

In response to this emerging and enduring trend of seeking healthy probiotic ingredients for skin beauty, with the support of extensive R&D, we have developed the first probiotic based on a native skin bacterium for anti-aging cosmetics. This probiotic is a beneficial skin-own *L. crispatus* that specifically decreases on skin face and in wrinkles during aging.

SKIN AGING FEATURES AND CAUSES

Skin is a large and complex organ with a primordial barrier function of protection of internal organs from harmful stressors, such as chemicals, pathogens, cold, heat, and ultraviolet radiations (UVR) (Park *et al*, 2021). However, the importance of the skin goes much further, encompassing an undeniable socio-cultural role. In fact, skin appearance and shape are of crucial importance to an individual's self-esteem, its care and beautification being part of the daily routine since ancient times (Favas *et al*, 2022). The delay of the skin-aging process has been a main societal demand. The loss of density and elasticity, the appearance of wrinkles and hyperpigmentation disorders are among the noticeable signs of skin aging (Park *et al*, 2021); these features of skin aging appearance are accelerated and worsened by ultraviolet (UV) radiations (Bacqueville *et al*, 2020).

Loss of dermal density with skin aging

Loss of dermal density with aging is linked to dermis depletion in extracellular matrix (ECM) components (Bae *et al*, 2017). Collagens which represent the major components of the dermis (Bae *et al*, 2017) decrease and their organization is impaired (Hiebert *et al*, 2011) (Figure 1). Collagen's decrease is observed in chronological or intrinsic aging as well as in extrinsic aging induced by environmental stressor namely UV. The mechanisms underlying collagen decrease with aging are, on one hand, a decrease in the capacity of the fibroblasts to produce them and on the other hand, an increase in the secretion of metalloproteinases or MMPs that destroy dermal collagen fibers (Shin *et al*, 2019, Favas *et al*, 2022).

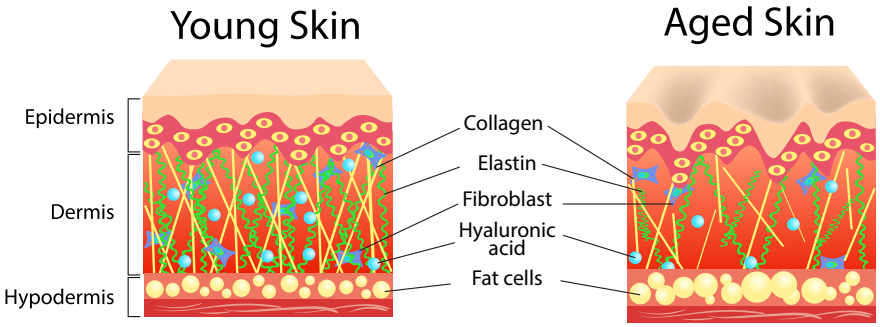

Figure 1 - Schematic representation of young and old skin.

In the skin, collagen fibers form higher-order networks that constitute the major dermal structure responsible for the skin's biomechanical properties (Ribeiro *et al*, 2013). Collagen I is the major fibrillar collagen in the dermal connective tissue and represents approximately 80%-90% of the total amount of the collagens synthesized by fibroblasts. Dysregulated collagen I synthesis at any step leads to diseases such as skin fibrosis (Horstmeyer *et al*, 2005) and loss of skin biomechanical properties like tensile strength and resistance to mechanical shock observed in old skin. Besides its contribution in skin biomechanical properties, collagen I is known to provide a structural scaffold for cell attachment with impacts on tissue organization and tissue homeostasis by affecting cell growth, motility, viability, and differentiation (Plant *et al*, 2009). Zôller and coworkers have shown that collagen I is also able to modulate lipogenesis and adiponectin expression and therefore may contribute to metabolic dysfunctions associated with aging (Zôller *et al*, 2019).

Type V collagen (COLV) is a minor component of collagen fibers and is found between Collagen I and Collagen III fibrils. Only its amino-terminal portion, which regulates the diameter of heterotypic fibers, projects to the outer surface (Roulet *et al*, 2007). Thus, this collagen contributes to the development of functional connective tissues (Martin *et al*, 2012). Collagen V regulates collagen fibrillogenesis; some data support a model whereby the collagen V:I ratio in different tissues determines the initial diameter and number of collagen fibrils assembled (Sun *et al*, 2015).

Consequently, the stimulation of collagen I and collagen V is a relevant target in order to increase the density of the dermis, and ultimately to limit the appearance and deepening of wrinkles.

Wrinkles appearance with skin aging

The facial wrinkles are the most apparent characteristic of aging (Nilforoushzadeh *et al*, 2022). Skin wrinkling is due to progressive loss of ECM components like collagens over time. There are other factors causing these wrinkles of the face, including constant pull of gravity, frequent and constant positional pressure on the skin of the face (e.g., during sleep), and repeated facial movements caused by contractions of mimetic muscles of facial expression (Fujimura *et al*, 2012).

Dermatologists can distinguish between two types of facial wrinkles: static and dynamic. Static wrinkles are always visible even when all facial muscles are resting as they developed in thin stretched skin as a result of premature or natural aging processes (Sun *et al*, 2015). However, with aging, these wrinkles become visible even at rest. Dynamic wrinkles, occurring in people of all ages even young children, appear when muscles contract causing the overlying skin to crease like an accordion. Accordingly, dynamic wrinkles are seen when persons are animating their facial expression (El-Domyati *et al*, 2014). To reduce facial wrinkles, and particularly the dynamic wrinkles, some people go for esthetic technics like lasers, toxins (Sun *et al*, 2015) or cold therapy (Palmer *et al*, 2015). For this reason, research on ingredients that may decrease dynamic wrinkles is still of interest to offer non-medical alternatives to consumers.

Oxidative stress, a major cause of skin aging

Reactive oxygen species (ROS) play an important role in skin aging (Choi *et al*, 2016). Intracellular and extracellular oxidative stress initiated by ROS advance skin aging (Masaki *et al*, 2010).

It is widely accepted that:

- intrinsic aging is primarily caused by accumulated damages due to free radicals and by ROS-induced damage to critical cellular macromolecules, like DNA and cellular membrane (Choi *et al*, 2016),
- the generation of ROS by UV radiation is one of the mechanisms through which UV light induces detrimental effects on skin named photoaging (De Jagger *et al*, 2017).

Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of ROS in cells and tissues and the decreased ability of a biological system to detoxify these reactive products (Pizzino *et al*, 2017) (Figure 2). ROS induced by UVB exposure increase the expression of matrix metalloproteinase-1 (MMP-1) in fibroblasts, promoting degradation of collagen type 1, which is an ECM component that provides structural support to the skin (Ha *et al*, 2019). This leads to a disintegration of the dermis, a decrease in dermis density and an acceleration of skin aging (De Jagger *et al*, 2017).

Not only does ROS production increase with age but the ability of human skin cells to repair DNA damage steadily decreases over time (Choi *et al*, 2016). The use of antioxidants is an effective approach to prevent symptoms related to aging and photo-induced aging of the skin (Masaki, 2010).

Skin possesses defense mechanisms against oxidative stress ranging from enzymes like superoxide dismutase, catalase, peroxiredoxin, and glutathion peroxidase to organic compounds such as L-ascorbate, tocopherol, beta-carotene, uric acid, CoQ10, and glutathione (Rinnerthaler *et al*, 2015).

A main contributor to the anti-oxidative potential of the cell is the tripeptide glutathione (GSH).

The GSH acts as an antioxidant because of its thiol group. GSH is oxidized by reactive oxygen radicals and forms a dimer with another activated GSH via formation of a disulfidic bond (GSSG). GSH can be recovered in a reducing step by the glutathione reductase consuming NADPH (Aung-Htut *et al*, 2012). GSH not only detoxifies ROS but can also regenerate oxidized tocopherol and retinol (Masaki *et al*, 2010). It is estimated that in aged skin the concentration of antioxidants is strongly decreased, in line with this, the levels of tocopherol, ascorbate and GSH have been shown to be reduced by 70% (Rhie *et al*, 2001).

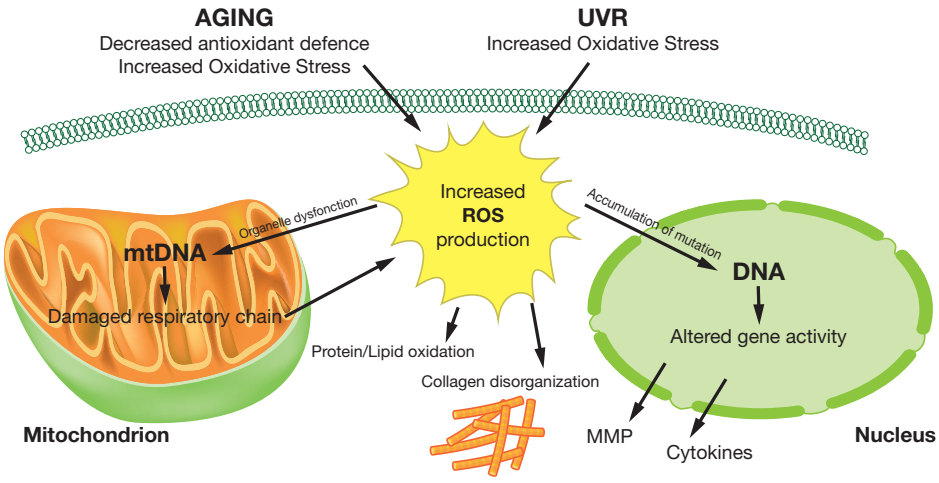

Figure 2 - Oxidative stress and skin aging (adapted from Davinelli *et al*, 2017).

PROBIOLIFT, NATURAL PROBIOTIC FROM THE SKIN YOUTH BACTERIUM *LACTOBACILLUS CRISPATUS*

L. crispatus, a youth bacteria identified from skin microbiota

Skin microbiome is more and more studied in dermatological field to develop solutions for different skin conditions. Indeed, several studies have harnessed that an imbalance of skin microbiota is associated with various skin disease such as atopic dermatitis, psoriasis and acne (Juge *et al*, 2020; Fourniere *et al*, 2020, Paetzold *et al*, 2019).

Although skin microbiome is considered an important component in skin health (Swaney *et al*, 2021), there was almost no research on the relationship between its composition and skin aspect particularly aged and wrinkled skin.

In our aim to explore skin microbiota and its evolution over time, we have conducted an exploratory study to compare facial skin microbiota of a young cohort aged from 18 to 35 yo with wrinkle grade of 0-1 to that of an old cohort aged above 55 yo with wrinkle grade 5-6. A particular focus was made on the wrinkle zone. Microbial samples were taken from 3 different zones: within the wrinkle hollow, the crow's feet and undereye zone, and the cheek area adjacent to the earlobe as control. DNA were then extracted from the samples and sequenced using Whole-Genome Sequencing protocols and taxonomic analysis was performed using available database.

The results confirm already reported data about the shift in the skin microbiota diversity and some species on old skin, (Shibagaki *et al*, 2017; Dimitriu *et al*, 2019). Analysis results also revealed that in the old cohort compared to the young one, there was a decrease in the Lactic acid bacteria namely Lactobacilli abundance and prevalence in the sampled zone (Figure 3 and 4). In older skin, among the 4 more abundant lactic acid bacteria present, *L. crispatus* was one of the most decreased especially in undereye zone and was absent in the wrinkle hollow (Figure 3 and 4). These results evidenced that aged skin is correlated to some changes in skin microbiota composition, particularly a decrease in prevalence and abundance of *L. crispatus*. This allowed us to hypothesize that *L. crispatus* is a youth bacterium and its use as a probiotic could be beneficial to fight skin aging.

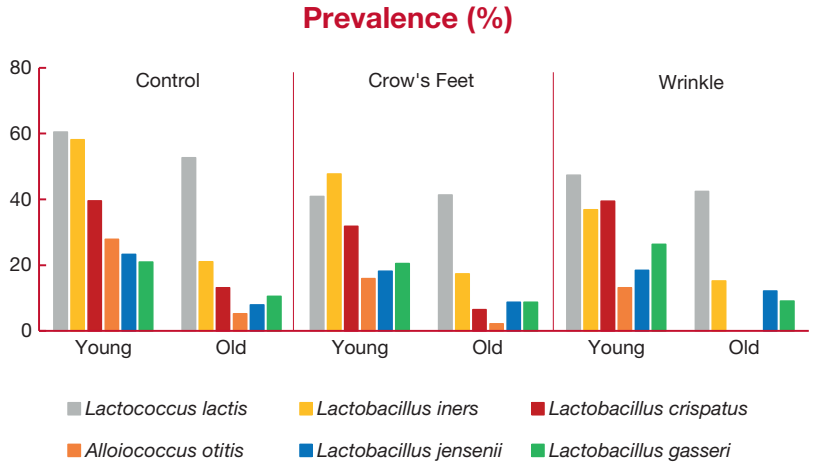

Figure 3 - Lactic acid bacteria prevalence in wrinkles area of the face of young cohort compared to that of old cohort.

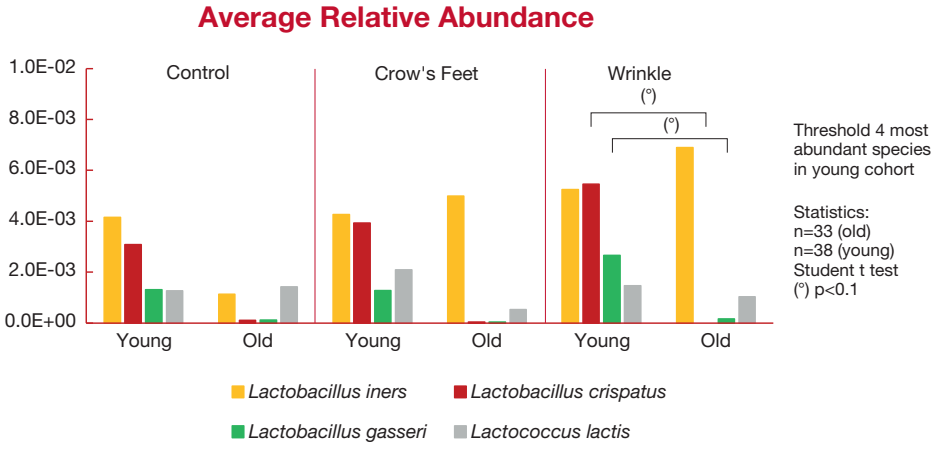

Figure 4 - *Lactobacillus* abundance in wrinkles area of the face of young cohort compared to that of old cohort.

For centuries probiotics have been used in food for improving gastrointestinal health (La Khmaladze *et al*, 2019). In recent years there has been a renewal of consumer interest in probiotics thanks to reported proven efficacy and due to the fact that they are considered as natural. Probiotics are increasingly used in the treatment of inflammatory and infectious pathologies of the intestine and certain mucous membranes such as the vaginal mucosa (La Khmaladze *et al*, 2019; Stravopoulou *et al*, 2020) most of the time through oral administration.

Lactobacillus species show a metabolic versatility and several exploitable functional properties which make them good candidate as probiotic or relevant for food fermentation to develop functional food (D'Alessandro *et al*, 2021).

Previous studies have reported the beneficial effects of lactic acid bacteria, namely Lactobacillus, their extracts or ferments on skin health, including improvements in skin conditions and the prevention of skin diseases (Huang *et al*, 2022). Thus, Lactobacilli derived product have been used in skin care products. Several *in vitro* and *in vivo* studies show that Lactobacillus is a genus that has been proven to hydrate the skin improving the barrier function, reduce inflammation and improve the entire functioning of the skin (Cinque *et al*, 2017; La Khmaladze *et al*, 2019; Lee *et al*, 2015; Tsai *et al*, 2021).

L. crispatus is a Gram-positive rod shape anaerobic bacterium. *L. crispatus* has been described as a natural inhabitant of some body mucosa (Pan *et al*, 2020). Its presence on the skin has been scarcely described.

As other lactobacillus, *L. crispatus* has been reported to show some valuable and host beneficial properties even if it is less used than other like *L. plantarum*. *L. crispatus* have shown *in vitro* and *in vivo* antimicrobial activities through its metabolites (Abdul-Rahim *et al*, 2021), some immunomodulatory and anti-inflammatory properties (Wang *et al*, 2021); as well as potential use in functional food (Siroli *et al*, 2017). Interestingly biosurfactant produced by *L. crispatus* have improved skin permeation of some drug (Abruzzo *et al*, 2021).

Probiolift, *Lactobacillus cripatus* probiotic isolated from human skin and obtained by fermentation

Probiolift is one of the two newly developed biotic ingredients Probiolift and Postbiolift to support graceful and healthy skin aging. Unlike other biotics existing on the market, the newly developed actives are the first to use a bacterium naturally present on the skin: *L. crispatus*, a bacterium which has been found to decrease with age.

L. crispatus strain used to develop Probiolift has been isolated from healthy human skin. After swabbing the skin of volunteers and successive plating and scraping on MRS (Man Rugosa Sharp) agar plate enriched medium (with aminoacids and carbohydrates), the isolated strains was identified by PCR. This strain was a wild type, not genetically modified. Isolated strain safety was assessed through its genome analysis. Virulence analysis was based on its full genome using Virulence Factor Database (VFDB). The results of this analysis showed that *L. crispatus* strain was non-pathogenic, non-biofilm forming, non-sporulating and non-toxin producer. No antibiotic resistance has been evidenced. When spread on a surface, cleaning and decontamination can be achieved using 0.5% Hypochlorite solution or 70% ethanol. This strain as other lactobacilli is classified Biosafety Level 1 (BSL1).

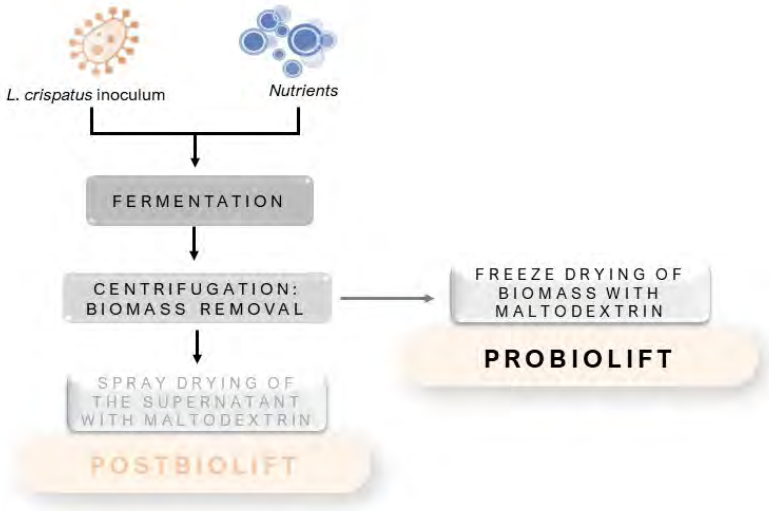

Figure 5 - Schematic process of Probiolift.

The 2 products Probiolift and Postbiolift are simultaneously produced from the same fermentation process: Probiolift, the probiotic with dormant cells of living *L. crispatus* (biomass) and Postbiolift, the supernatant containing the metabolites secreted by *L. crispatus* (Figure 5). With this approach we ensure that no additional resources is required to produce the 2 products, inputs are mutualized during the process, in addition we also minimize the production of waste (as the non-valorized part would have been discarded).

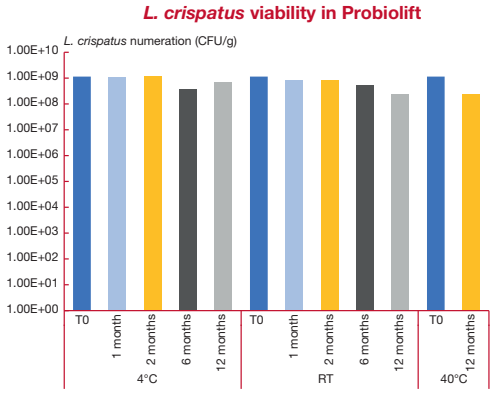

Figure 6 - Viability of *L. crispatus* over time at 4°C, RT and 2 months at 40°C expressed in colony forming units per gram (cfu/g).

The fermentation conditions of *L. crispatus* were optimized in terms of temperature, pH, fermentation medium and nutrients. Highest production of viable *L. crispatus* biomass was obtained under these optimized conditions with total consumption of the carbon source nutrient. After centrifugation to retrieve *L. crispatus* biomass, it was then mixed with maltodextrin as cryoprotectant and freeze dried to give Probiolift as a powder (INCI: Maltodextrin, Lactobacillus) characterized by more than 1 million of viable *L. crispatus* bacterial cells expressed in Colony Forming Unit (CFU) per gram. Use of probiotic for application is a challenge because of possible loss of viability during conditioning and storage. With Probiolift, we checked bacterial *L. crispatus* viability over one year of storage at 4°C and room temperature (RT), and over 2 months at 40°C (Figure 6). In all these conditions, we did not observe any significant loss of viability over time.

Formulation

The use of probiotics in topical cosmetic is often challenged by the decrease in their viability over time in such formulations. It is challenging for the cosmetic industry to create topical formulas that retain probiotic bacterial viability from production to the value chain and onto the consumer (Puebla-Barragan and Reid, 2021). Some ingredients basically used in aqueous based cosmetic formulations lead to a decrease in the viability of probiotics, which in turn decrease their effectiveness. This phenomenon is observed, among others, with the preservatives used to protect formulations from microbial contamination. Thus, oily based or anhydrous formulations appeared to us as the most suitable formulation for Probiolift application to also fit into the trends of solid cosmetics of skincare and make-up. After screening the compatibility and the stability in terms of viability of *L. crispatus* strain in 30 liposoluble or lipodispersible raw materials, a set of 6 anhydrous formulations including serum, stick and make-up powder were developed with *L. crispatus* probiotic incorporated at adapted temperature up to 70°C. The stability and the viability of *L. crispatus* was monitored at 4°C and room temperature

Depending on the galenic formulation and the temperature, Probiolift viability was ensured from 3 months to 12 months (Figure 7).

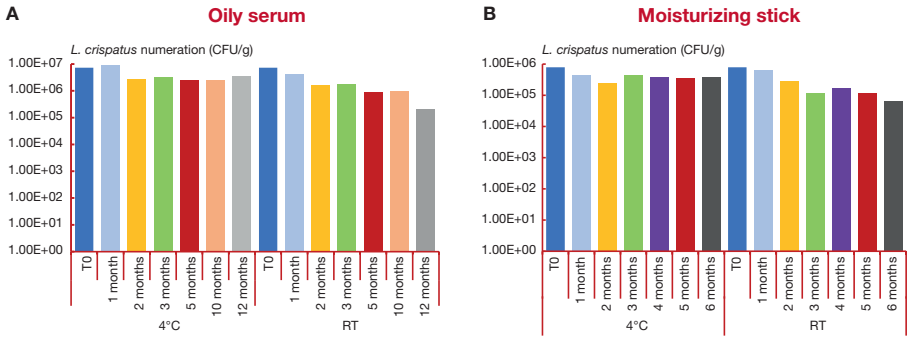

Figure 7 - Viability/stability of *L. crispatus* over time in oily serum (A) and in a moisturizing stick (B).

Finally, if an emulsion formulation is required for the application, we have proven that it is possible to develop such galenics using a commercially available dual chamber container, where an oily serum containing Probiolift is stored in a separate container from an aqueous emulsion. Both are available simultaneously by pressing on the pump. In such conditions *L. crispatus* viability can be maintained at least 3 months at room temperature until 5 months at 4°C (data not shown).

Safety / tolerability of the product

Probiolift was tested to ensure its safety under the recommended conditions of use without any indication of skin irritation or sensitization.

Description

Probiolift is a skin probiotic, made by dormant, living cells of *L. crispatus*, a beneficial microorganism naturally occurring on young skin. It is available as a preservative-free powder.

INCI name: Maltodextrin (and) Lactobacillus

Naturalness content (according to ISO 16128): 100% from natural origin

Dose of use: 0.05%

China IECIC listed

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Example: illustration of a commercial sample of Probiolift, with an oily serum and a stick containing it at 0.05%.

DEMONSTRATED EFFICACY

Probiolift efficacy was tested *in vitro* and *in vivo*. *In vitro,* it showed anti-aging properties thanks to its activities on collagen synthesis and oxidative stress. It has been proven clinically to improve skin density, to decrease forehead wrinkles, helping thus the skin to go for a graceful aging.

***In vitro* performance**

Evaluated on fibroblasts monolayer culture, Probiolift proved its efficacy *in vitro* to stimulate dermal components:

- it increased collagen I synthesis, the major component of skin dermis by fibroblasts, contributing thus to an increase in dermal density,
- it boosted collagen V synthesis by fibroblasts, thus contributing to a proper collagen fibers organization.

Probiolift showed potent antioxidant properties:

- it showed a high free radical scavenging capacity.
- at cellular level, Probiolift showed a capacity to decrease the effects of UV-induced oxidative stress by decreasing the level of cellular ROS and lipid peroxidation. At the same time, it significantly increased the level of the endogenous antioxidant glutathione.

Interestingly, Probiolift contributed to skin relaxation by decreasing contraction frequency of neuromuscular cell culture *in vitro*; this activity may contribute to a decrease in wrinkle appearance.

***In vivo* performance**

Postbiolift demonstrated its ability to increase dermal density and to reduce forehead wrinkles thickness.

EFFICACY

Stimulation of extracellular dermal components: collagen I and collagen V synthesis by fibroblasts

in vitro

OBJECTIVES

During aging, skin density decreases due to decrease in ECM components namely collagen, and collagen fibers organization impairment.

The aim of this study was to assess Probiolift capacity to enhance the synthesis in dermal fibroblasts monolayers of collagen I and collagen V involved in collagens' fiber organization. This quantification was made using Delfia method to quantify extracellular deposited collagen I and V.

In addition, we compared the efficacy of the viable cells, Probiolift, with the non-viable inactivated and lysate forms of the *L. crispatus* biomass.

RESULTS AND DISCUSSION

Vitamin C (ascorbic acid), a well-known enhancer of procollagen hydroxylation and secretion, was used as a positive control. It significantly increased collagen I and V synthesis and deposition (data not shown) by fibroblasts, allowing us to validate the experiment.

The results obtained with Probiolift showed a dose-dependent and significant stimulation of collagen I synthesis and deposition by fibroblasts, by +133% at 0.125% and +179% at 0.25% ($p<0.05$) (Figure 8).

Probiolift also turned out to have a dose-dependent and significant effect on collagen V production by fibroblasts, by +55% at 0.125% ($p<0.05$) and +68% at 0.25% ($p<0.01$) (Figure 9). Collagen V plays a crucial role in the 3D organization of collagen I and is thus of major importance for collagen network functionality and as a consequence, for skin biomechanical properties also.

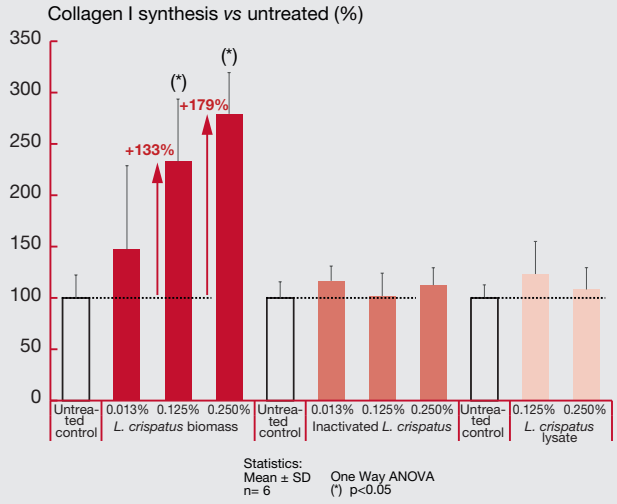

Figure 8 - Effect of Probiolift, inactivated and lysed *L. crispatus* biomass on collagen I synthesis on fibroblast monolayer.

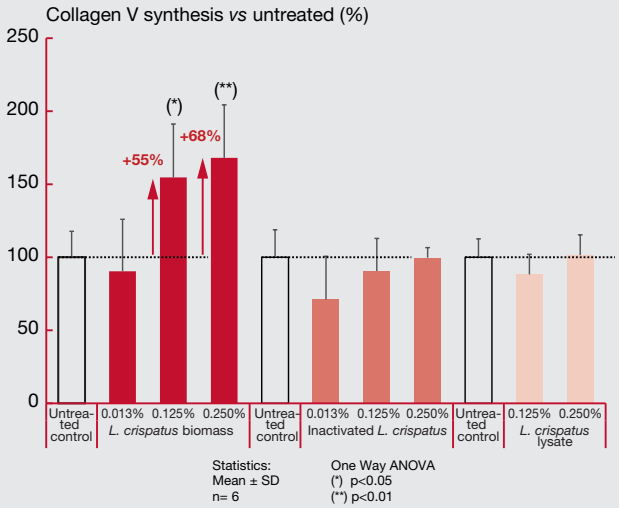

Figure 9 - Effect of Probiolift, inactivated and lysed *L. crispatus* biomass on collagen V synthesis on fibroblast monolayer.

Inactivated *L. crispatus* biomass was obtained after heating while lysed *L. crispatus* biomass was obtained by sonicating of a suspension of *L. crispatus* biomass. When tested in the same condition as Probiolift, lack of collagen I and collagen V syntheses stimulation was evidenced for the inactivated and lysed biomass. This suggests strongly that collagen I and V stimulation capacity is related to the fact that *L. crispatus* is alive in Probiolift.

CONCLUSION

These results showed the ability of Probiolift, to increase the synthesis of collagen I and V. Thus, Probiolift may contribute to improve collagen fibrils formation and density in the dermis.

MATERIALS&METHODS

Cell culture

Normal human dermal fibroblasts obtained from a 19-year-old donor were cultured in monolayers and grown to confluence in 96 well-plates, thereafter incubated 48h at 37°C and 5% CO_2 in the presence of the ingredients to be tested.

Products:

Vitamin C or ascorbic acid was purchased from Sigma.

Probiolift containing viable biomass was obtained thanks to the process described page 8.

Inactivated biomass was obtained after fermentation and centrifugation steps previously described (page 8), the viable biomass pellet is resuspended in fermentation medium and thermally inactivated by heating at 80°C for 30 minutes.

Lysed biomass was obtained after suspension of 1% of Probiolift in distilled water, sonication 10 minutes, freezing (-80°C) and thawing (37°C). This process allowed us to perform lysis of the bacterial cells and to extract the intracellular bacterial cells content. Before application the suspension was filtered at 45µm.

Treatments

Vitamin C (ascorbic acid) at 50µM was used as positive control. The untreated control was represented by fibroblasts culture medium. The candidates were tested at concentrations which didn't induce cell cytotoxicity. Probiolift or thermally inactivated biomass of *L. crispatus* were suspended in Phosphate Buffer Saline (PBS) first, then diluted in FGM medium and incubated with fibroblasts at following final concentrations 0.013%, 0.125% and 0.25% (w/w) during 48h. Lysed biomass solution was tested at 0.125% and 0.25% (w/w).

Collagen I and V quantification by Delfia method

After incubation in the presence of the tested products, the medium was discarded, and a dedicated lysis solution was added. This solution allows to disrupt cell membranes without solubilizing the deposited matrix. Then, the lysis buffer was removed and replaced by a PBS / Bovine Serum Albumin (BSA) saturation solution (Perkin Elmer, Courtaboeuf, France). The primary anti-collagen I or anti-collagen V antibodies (Interchim, Montluçon, France) were added. After rinsing in PBS, the Europium-conjugated secondary antibody (Perkin Elmer) was added. Finally, a specific enhancement solution (Perkin Elmer) was added. Fluorescence intensity was read (λexc. 340 nm / λem. 615 nm) using a spectrophotometer (Perkin Elmer).

Results and statistics

Results are expressed as mean % of collagen I or collagen V synthesis *vs* untreated control. The statistical comparison was performed running Student t test for vitamin C (data not shown) and untreated control comparison and using One way ANOVA method for Probiolift, inactivated, lysed *L. crispatus* biomass and untreated control comparison.

EFFICACY

Skin relaxation: inhibition of subcutaneous cell contraction

in vitro

OBJECTIVES

Expressional wrinkles appear on the skin at around 30 years old, on the face where skin have to adapt to the facial muscle's movements and gravity. Facial movements are based on repeated subcutaneous muscle contraction and create with aging expressional wrinkles which are difficult to remove. The goal of this experiment was to evaluate Probiolift capacity to decrease the frequency of cell contraction, in a model of motor neurons / muscle cell co-culture.

RESULTS AND DISCUSSION

After 2, 8 and 24 hours of incubation, the initial contraction frequency was stable for the untreated condition (without any product) compared to the beginning of the recording (dotted line: basal level of contraction at T0).

The positive control α-bungarotoxin at 1µM significantly inhibited cellular contraction starting at 2 hours of incubation; this result validated the experiment.

Probiolift induced a significant inhibition of the contraction frequency as early as 2 hours after treatment at 0.025% (Figure 10). The significant effect observed from two hours persisted after 24 hours of incubation.

Probiolift observed efficacy is comparable to that of the α-bungarotoxin positive control of inhibition which acts by binding specifically to acetylcholine receptors.

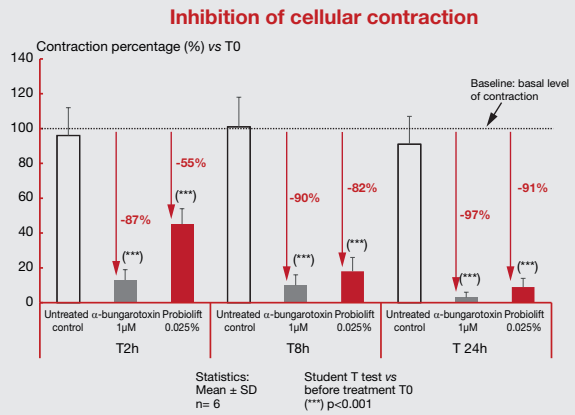

Figure 10 - Effect of Probiolift on cellular contraction frequency.

CONCLUSION

Probiolift showed a significant inhibition of cell contraction, and thus its potential for an improved skin relaxation to lower the appearance of expression wrinkles.

MATERIALS&METHODS

Cell culture

Human Induced Pluripotent cells (hiPS) derived from reprogrammed human fibroblasts were seeded in 6-well plates coated with a thin layer of Matrigel (Dutscher), in a growth medium for hiPS cells. This medium was then changed by a succession of 3 differentiation media. These media are supplemented with different factors inducing a specific differentiation towards the motor neuron pathway. The cells were maintained in culture at 37°C and 5% CO_2 for 10 days.

After 9 days of differentiation in the different culture media, the differentiating hiPS cells were recovered and seeded in 96-well plates on top of a mat of human muscle cells from an adult donor seeded 24 hours before on a Matrigel coated medium. These cells grew in the differentiation medium.

Under these conditions, after 3 weeks of co-culture, the muscle cells contract spontaneously under the impulse of the motor neurons. These contractions can be blocked by α-bungarotoxin at 1µM (Santhanam *et al*, 2018).

Cell treatment and contraction registration

At this stage, the cells were placed under an InCell 2200 automated microscope (GE Healthcare) in a controlled atmosphere at 37°C. For each culture well, the areas where muscle fibers are contractile were identified; on all identified areas, a 30-second movie and cells 's contraction frequency were recorded (basal contraction conditions).

At the end of the first recording, the compounds or α-bungarotoxin were incubated. The following treatment conditions were performed:

- control medium,
- control medium + active matter equivalent to Probiolift at 0.025% (w:w),
- control medium + α-bungarotoxin at 1µM as positive control.

Each condition was repeated 6 times.

By experimental condition, contraction frequencies were analyzed before treatment (basal level of contraction) and then after 2 hours, 8 hours, and 24 hours of treatment.

Statistics

Results were expressed as mean ± SD percentage of contraction frequency which was calculated relative to the frequency of contractions before incubation with tested compounds (basal level of contraction). The basal level of contraction condition was considered as the maximum frequency (100%). Intergroup comparisons were performed by an unpaired Student t test; The threshold of significance was set to 5% ($p<0.05$).

EFFICACY

Antioxidant capacity

Free radical scavenging capacity

in vitro

OBJECTIVES

Oxidative stress is one of the major causes of aging. The goal of this test was to assess the capacity of Probiolift to inhibit oxidative stress by scavenging free radical such as the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH°).

RESULTS AND DISCUSSION

The experiments were validated thanks to the free radical DPPH° inhibition observed with the positive control Vitamin C at 1mM (100% of inhibition, $p<0.001$, data not shown).

Probiolift starting at 0.05% scavenged significantly the number of free radical DDPH° with a dose-dependent efficacy and an almost total scavenging effect at 0.25% dosage (-98%, $p<0.001$) (Figure 11).

When tested at the same dose, the corresponding thermally inactivated and lysate of *L. crispatus* biomass didn't show any scavenging effect on DPPH° radical; this suggest strongly that the observed free radical scavenging capacity of Probiolift is linked to the viable state of the *L. crispatus* strain in Probiolift.

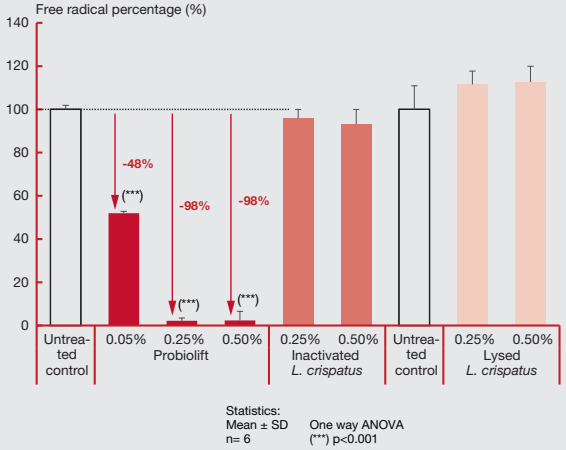

Figure 11 - Effect of Probiolift, inactivated and lysed *L. crispatus* biomass on DPPH° free radical scavenging.

CONCLUSION

Probiolift effectively scavenged DPPH° free radical. These results indicate the potential of Probiolift to fight against reactive oxygen species generated by oxidative stress and thus to protect the skin from oxidative stress, a major cause of skin aging.

MATERIALS&METHODS

Material

2,2-diphenyl-1-picrylhydrazyl or DPPH° from Sigma was used as free radical.

Vitamin C was also purchased from Sigma.

Free radical quantification

100µl of an ethanolic solution of DPPH° at 1mM was mixed with 100µL of Phophate Buffer Saline (PBS) suspension of the ingredients to be tested in 96 well plates. Probiolift was tested at final concentrations of 0.05% to 0.5% (w/w). Thermally inactivated and lysed biomass of *L. crispatus* prepared as previously described were tested in the same conditions at the following final concentrations of 0.25% and 0.5%.

The untreated control for ingredients was made using the strain's dissolution solvent, PBS and a positive control was made with aqueous 1 mM vitamin C solution. The corresponding untreated control of Vitamin C was water.

Each of the solutions containing the products, the untreated control or the positive control was mixed at 50%/50% (v/v) with an ethanol solution which is the solvent for DPPH°, in order to produce a blank, in this same plate and deduct interference of the tested ingredients 'absorbance.

The whole was incubated for 30 minutes in the dark under stirring. At the end of the incubation, the optical density (OD) was measured at a wavelength of 530 nm to estimate the amount of free radicals formed under each tested conditions.

Results and statistics:

Results were expressed as mean average percentage of free radical ± SD of trial made in n=6. The untreated control was considered as maximum rate of radical formation equivalent to 100%.

The free radical scavenging capacity was then deducted by the difference of radical percentage between untreated and the tested condition.

The statistical analysis was conducted by a One Way ANOVA method for the ingredients and by a student test for vitamin C. The threshold of significance was set to 5% ($p<0.05$).

EFFICACY

Antioxidant capacity

Inhibition of the cellular effect of UV-induced oxidative stress

in vitro

OBJECTIVES

Intracellular and extracellular oxidative stress initiated by reactive oxygen species (ROS) advance skin aging (Masaki *et al*, 2010). The generation of ROS by UV radiation is one of the mechanisms through which UV light can manifest its possible detrimental effects on skin named photoaging (De Jager *et al*, 2017). To assess the effect of Probiolift on cellular UV-induced oxidative stress, we used fibroblasts monolayer culture on which UVA rays were used to generate an oxidative stress. Then Probiolift effect on intracellular ROS level, cellular antioxidant defense (Glutathione, GSH) level and lipid peroxidation was evaluated.

RESULTS AND DISCUSSION

Vitamin E used as positive control inhibited significantly (-29%; $p<0.05$) the intracellular ROS formation after UVA and also decreased significantly by 52% ($p<0.001$) the cellular lipid peroxidation induced by UVA, thus validating these experiments (data not shown).
Probiolift at 0.0062% significantly decreased cellular ROS level by 50% after UVA exposure ($p<0.05$) (Figure 12A).
Interestingly and unexpectedly, Probiolift at 0.0062% increased level of glutathione by 65% ($p<0.05$) in fibroblasts (Figure 12B). Glutathione is an endogenous antioxidant molecule often used in cosmetic product for these properties. So far, today on the market there are very few products claiming a stimulation of glutathione synthesis.
Finally, Probiolift at the same concentration, 0.0062% decreased significantly by 32% ($p<0.05$) the level of lipid peroxidation induced by UVA radiations (Figure 12C) thus limiting their negative impacts on lipids from cell membranes.

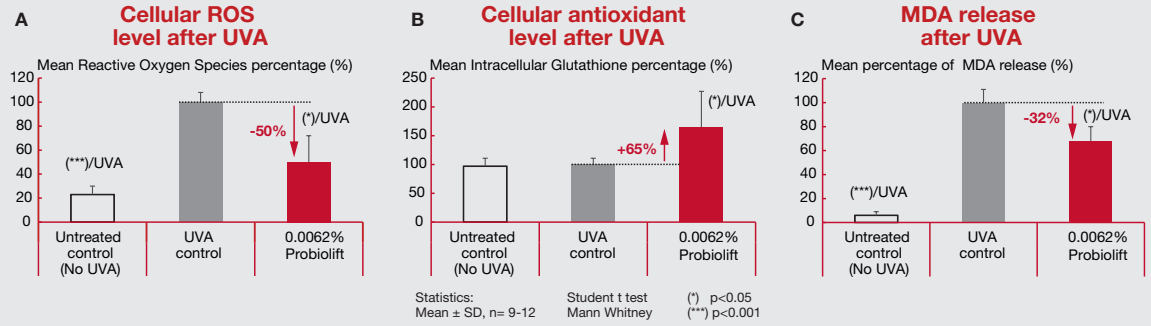

Figure 12 - Effect of Probiolift on UVA-induced cellular oxidative stress. **A**- Effect on cellular ROS level. **B**- Effect on cellular antioxidant (glutathione level, GSH). **C**- Effect on lipid peroxidation (malondialdehyde level, MDA).

CONCLUSION

By decreasing cellular ROS production, lipid peroxidation and increasing cellular level of glutathione after UVA irradiation, Probiolift effectively limited the deleterious effects of UVA on skin fibroblast cells. These results show Probiolift potential to protect skin cells against UV-induced oxidative stress and generally to protect skin against oxidative stress which is one of the major causes of skin aging.

MATERIALS&METHODS

Cell culture

Normal human fibroblasts, from a healthy donor were seeded in 24-well plates at a rate of 30,000 cells/cm², then cultured in DMEM (Dulbecco's modified Eagle's minimum essential medium)/Ham's F-12 medium, supplemented with 10% fetal calf serum (FCS) and 0.5% antibiotic, for 96h at 37°C, under 5% CO_2 and 95% relative humidity.

Treatments

0.5mL of the cell suspension was incubated 72h in the presence of the ingredient to be tested in EMEM (Eagle's minimum essential medium) supplemented with 1% FCS:

- Probiolift was tested at a final concentration in well of 0.0062% (w/vol),
- vitamin E (Tocopherol) used at 0.0003% (v/v) as positive control for inhibition of lipid peroxidation and cellular ROS formation,
- the culture medium without addition of any product nor UVA ray was used as an untreated control The same condition with UVA irradiation was used as UVA control.

After the first hour of incubation at 37°C under 5% CO_2 and 95% relative humidity, the cells were irradiated with UVA at 20J/cm² for 3 hours; then the incubation was followed until 72h before cellular ROS quantification, and malondialdehyde (MDA) quantification as lipid peroxidation endpoint, and until 96h for glutathione quantification.

Oxidative stress quantification

Quantification of cellular ROS generation after UVA

A DCFH-DA (2',7'-dichlorodihydrofluorescein diacetate) probe solubilized at 10µM in PBS (phosphate buffer) was incubated with the cells before UVA irradiation. Quantification of cellular ROS released after UVA irradiation was made using the fluorescence readout on the cell monolayer related to the formation of DCF (2',7-dichlorofluorescein) from the probe (DCFH-DA). Fluorescence was recorded at 485 nm excitation and 538nm emission.

Quantification of cellular antioxidant molecule glutathione after UVA

96h after incubation, the irradiated medium was removed, and the cells were rinsed with PBS.

1N sodium hydroxide (NaOH solution) was added to each well, followed by a solution of o-phthaldialdehyde (OPT) diluted 1/15th in GSH (reduced L-glutathione) buffer. The whole was incubated for 15 minutes at room temperature. Then the amount of glutathione was measured by recording fluorescence at 355 nm excitation and 430 nm emission.

Quantification of lipid peroxidation after UVA

An amount of 0.5 mL of the supernatants was transferred to a pyrex glass tube to which a 40% trichloroacetic acid solution and 2% thiobarbituric acid were added. The resulting mixture was heated for 30 minutes at 100°C. Then, the formation of MDA (MalonDiAldehyde), which is an oxidation product released following UVA-induced lipid peroxidation, was measured by fluorescence at 532nm excitation and 560nm emission.

Results and statistics

Results are expressed as mean percentage of ROS, glutathione and MDA ± standard deviation of 9 to 12 replicates (n= 9-12). Results were compared to the UVA control normalized to 100% and statistically compared to each other using the Student t test if normality test passed or Mann-Whitney Rank Sum test if normality test failed. The threshold of significance was set to 5% ($p<0.05$).

In vitro performance conclusion

We demonstrated *in vitro* the antiaging and rejuvenating properties of Probiolift.

Activation of extracellular matrix component synthesis through:
- stimulation of Collagen I **(+133%, p<0.05)** and V **(+55%, p<0.05)** production in fibroblasts monolayer at 0.125%.

Subcutaneous cell relaxation by:
- the decrease in subcutaneous cells contraction frequency starting after 2 hours, with highest observed effect after 24h **(-91%, p<0.001)** of incubation at 0.025%.

Protection of skin and skin cells from oxidative stress through:
- free radical scavenging **(-48%, p<0.001)**,
- Inhibition at 0.0062% of intracellular ROS **(-50%, p<0.05)**, of lipid peroxidation **(-32%, p<0.05)** and increase in antioxidant glutathione level **(+65%, p<0.05)** after UVA-induced oxidative stress.

In summary, Probiolift showed *in vitro* some potential in rejuvenating the skin by increasing production of extracellular matrix components, by relaxing the subcutaneous cells and by protecting skin cell from oxidative stress.

EFFICACY

Improvement of skin density and wrinkles with Probiolift for a youthful skin

in vivo

OBJECTIVES

Skin changes are among the most visible signs of aging. With aging skin density decreases and wrinkles appear (Favas *et al*, 2022). The delay of the skin-aging process has been and is still a main societal demand.

In order to assess the effect of Probiolift on skin aging signs, skin density and skin wrinkles, a double-blind placebo-controlled clinical study was conducted on a panel of 29 healthy Caucasian volunteers.

29 female Caucasian volunteers aged 45-65 years old, having forehead fine lines / wrinkles and considering having loss of firmness
Randomized split face, twice daily self-applications for 56 days

Placebo  Probiolift 0.05%

Evaluation of total dermis density and SELEB density.
Evaluation of forehead wrinkles.
Time points: D0, D21 and D56.

Figure 13 - Clinical study design.

RESULTS AND DISCUSSION

1. Skin densification effect:

Ultrasound measurements were performed on the cheek, and the resulting images were analyzed for Total Dermis Density and Sub-Epidermal Low Echogenic Band (SELEB) density. SELEB is a consistent echostructural parameter of aged and photodamaged skin in the upper dermis. SELEB density is considered to closely reflect the degree of cutaneous aging (Gniadecka *et al*, 2014). An increase in Total Dermis Density corresponds to a restructuring of the fibrous network, and an increase in SELEB Density corresponds to a collagenous re-densification.

After 2 months of application, Probiolift at 0.05% significantly increased the total dermis density by 6% ($p<0.05$) compared to baseline whereas the placebo did not demonstrate any significant change. This increase was significantly higher compared to that obtained with the placebo (+5%, $p<0.05$ Figure 14A). These results on dermal density, illustrated by the example in figure 15, shows with Probiolift a densifying effect correlated to a restructuration of the fibrous collagen network.

On the SELEB band, after the first 3 weeks of application, Probiolift at 0.05% increased significantly the density by 9% ($p<0.05$) *vs* baseline whereas the placebo did not display any significant change (Figure 14B). After 2 months, the increase of SELEB density reached +11% ($p<0.001$) compared to baseline for Probiolift at 0.05%, which was better than for the placebo (+6%, $p<0.05$) even if the difference did not reach the statistical significance.

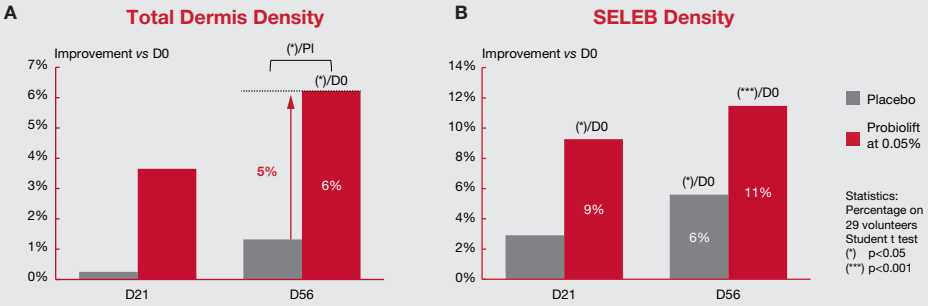

Figure 14 - Effect of Probiolift at 0.05% and placebo dermal (A) and SELEB density (B).

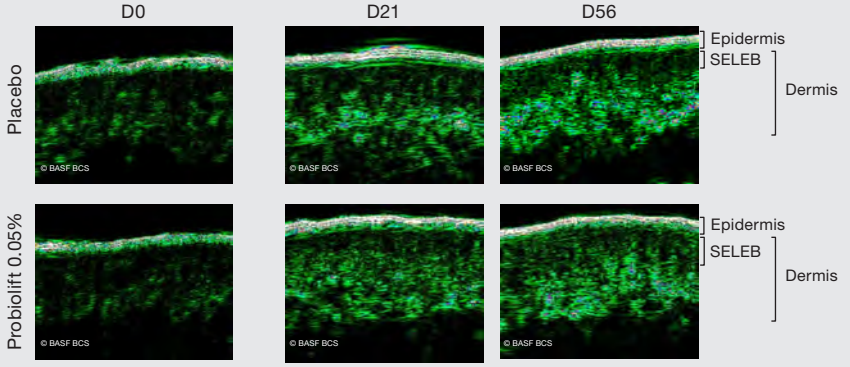

Figure 15 - Illustrative image of densifying effect of Probiolift on SELEB and Dermis. D0: baseline. D21 and D56 after 3 weeks and 2 months of Probiolift and placebo formulation application (Volunteer 10).

in vivo

2. Anti-wrinkle effect

After 2 months of application, Probiolift decreased significantly the forehead wrinkle thickness compared to the baseline and compared to the placebo formulation (-5%, $p<0.05$) as measured by VISIA image analysis (Figure 16).
This anti-wrinkle effect is also visible as shown in illustrative images in Figure 17.

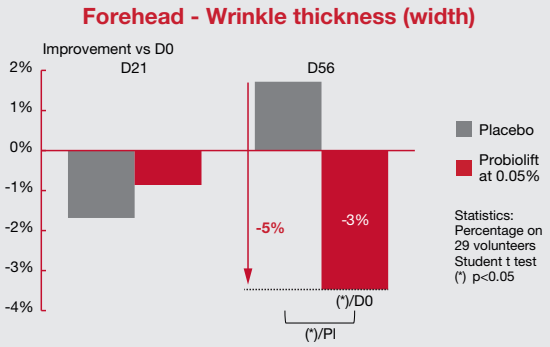

Figure 16 - Probiolift effect on forehead wrinkle thickness.

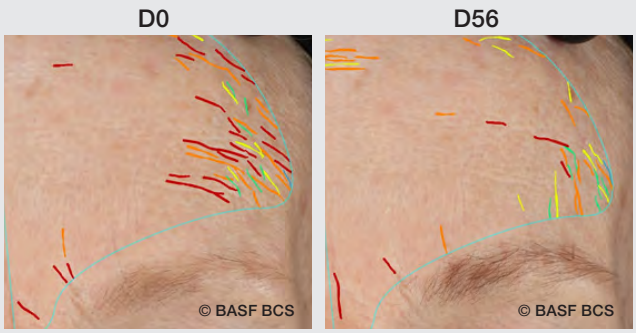

Figure 17 - Illustrative pictures on one volunteer of the improvement of the forehead wrinkle appearance with Probiolift at 0.05% D0: baseline (before treatment), and D56: after 56 days of formulation application.

Red and orange lines represent very coarse and coarse lines. Yellow represent medium lines. Blue and green lines represent fine lines. (Volunteer 27).

CONCLUSION

Probiolift at 0.05% improved the characteristics of skin aging with a dermis densification and a decreased forehead wrinkle appearance for a younger skin appearance.

MATERIALS&METHODS

Study design

The clinical study was carried-out as a randomized, double-blind, and split-face *vs* placebo. The efficacy of the formulation containing Probiolift at 0.05% was compared to the baseline (before treatment, D0) and to the placebo formulation. The formulation is detailed in Annex 2.
The study was conducted during a period of 56 days (2 months) with check points at D0, D21 and D56.

Inclusion criteria

The study was done on 29 healthy Caucasian female volunteers aged from 45 to 65 years old having fine lines/wrinkles on the forehead and a self-perception of loss of firmness, especially on their cheeks.

Application modality

For this study volunteers were given 2 products:
- an oily serum booster containing Probiolift at 0.42% and a microbiome friendly emulsion base, meaning that has been proven to be neutral to the skin microbiota,
- an oily serum booster placebo containing maltodextrin at 0.42% and the same microbiome friendly emulsion.

For Probiolift and Placebo, volunteers were told to put one drop of oily serum booster to two pumps of emulsion (to have Probiolift at 0.05% in the mix), to mix and then immediately apply the mix on the dedicated half face. Volunteers applied twice a day, once in the morning and once in the evening, for 56 days the Probiolift formulation at 0.05% or the placebo formulation on half face, under normal conditions of use. The last application was done the evening before the visit.

Evaluation methods

Skin density measurement by Ultrasound images and Image analysis.
The DUB-SkinScanner systems are high frequency and high-resolution diagnostic ultrasound systems. It allows *in vivo* non-invasive skin analysis.
The measurements were performed on cheeks (arbitrary unit :au) and image analysis were done on all the measurements. The following parameters were measured: SELEB density and total dermis density.
An increase in SELEB Density (arbitrary unit: au) corresponds to a collagenous densification. An increase in total dermis density (arbitrary unit: au) corresponds to a restructuring of the fibrous network.

Anti-wrinkle effect

The VISIA has a multi-point positioning system and ghost imaging capability that captures well registered images to document progress over time. The VISIA captures and automatically analyzes left, right and frontal facial views. The multi-spectral lighting includes IntelliFlash, cross-polarized and UV lighting to record and measure the surface skin condition. For this study, the VISIA images were analyzed by the corresponding Canfield VAESTRO image analysis software to assess forehead wrinkle thickness.

Statistics

Results are expressed as the mean percentage of change compared to the baseline measurement.
The statistical analysis of the parameters has been done after the verification of the normality of distribution using Shapiro-Wilk test.
The following tests were used:
- validation of the normality of the studied parameters: Student t test,
- invalidation of the normality of the studied parameters: Wilcoxon test.

GENERAL CONCLUSION

Probiotic for graceful aging

- First natural probiotic from the native skin bacteria *Lactobacillus crispatus*, which specifically decreases in wrinkles during aging.
- Part of the Biotic rejuvenation range, including also Postbiolift as postbiotic for healthy aging, based on youth bacterium.
- *In vitro*, stimulates synthesis of collagens, slows-down cell contractions and protects skin cells against oxidative stress.
- Clinically proven to reduce the forehead wrinkles appearance and to improve skin density.

There is an increasing interest in probiotics for the treatment of skin conditions. Despite some challenge in topical use of these living microorganisms, they are becoming more and more marketable and are acknowledged as natural and safe by consumers. Their use is now being extended to cosmetics.

At every life stage, and all over the world, there is a need for healthy looking skin. Thus, there is a need for ingredients able to decrease the signs of skin aging such as a decreased skin density and wrinkles appearance. In addition to that skin protection from oxidative stress is also required for a healthy and graceful aging.

After discovering that the skin native bacterium *L. crispatus* decreases with age, particularly in the wrinkle hollow, we have isolated this beneficial bacterium and developed Probiolift as a true living probiotic. Produced by fermentation, Probiolift contains more than one million of bacterial cells per gram. Probiolift' living bacteria were demonstrated to be stable and viable all over the process and under the storage conditions.

In vitro, Probiolift was shown to increase the synthesis by fibroblasts of the major skin collagen (collagen I) and also collagen V, an important collagen for collagenous fibers proper organization. Probiolift also showed to slow-down cell contractions. Finally, it showed strong antioxidant properties, by scavenging free-radicals, stimulating the anti-oxidative molecule glutathione and decreasing the effects on cells of UV-induced oxidative stress.

The benefits of application of this living native Lactobacillus on the skin were demonstrated in a placebo-controlled study on a panel of 29 volunteers. Probiolift showed an improvement of the total dermal density and a reduction in forehead wrinkle appearance.

Taken together, the objectivation results make Probiolift an innovation breakthrough leading the way for using topical probiotic for anti-aging cosmetic application.

ANNEXES

Annex 1 - Technical data - Available upon request

- Quality and Regulatory Product Information
- Toxicological abstract
- Composition sheet
- Specifications
- Formulation Data Sheet
- Natural content origin (according ISO 16128)
- Product Purity Profile

Annex 2 - Clinical test formulas

Emulsion base

Trade name	INCI name	Formulation %
Glycerin 99-5	Glycerin	5.00
1,3-Butanediol	Butylene Glycol	10.00
Rheocare XGN	Xanthan Gum	1.00
Water	Water	qsf 100
Sodium benzoate	Sodium Benzoate	0.25
Dermosoft 1388 Eco	Glycerin, Aqua, Sodium Levulinate, Sodium Anisate	1.00
Cutina HVG	Hydrogenated Vegetable glycerides	2.50
Cetiol CC	Dicaprylyl Carbonate	4.00
Cetiol RLF	Caprylyl Caprylate/Caprate	6.50
Plantaquat LC 7	Cetearyl alcohol, Lecithin, Sodium Cetearyl Sulfate, Olus oil	3.00
Citric Acid (50% solution)	Citric acid	0.40

Oily serum booster

Trade name	INCI name	Placebo formulation %	Probiolift formulation %
Cetiol C5	Coco Caprylate	99.58	99.58
Glucidex 6	Maltodextrin	0.42	-
Probiolift BC 10157	Maltodextrin, Lactobacillus	-	0.42

Annex 3 - Formulation examples

Live probiotic night booster (SC-FR-22-BC-50930-02)

Phase	Ingredients	INCI	% by weight	Function
A	Myritol® 318	Caprylic/Capric Triglyceride	39.08	Emollient
	Cetiol® C 5C	Coco-Caprylate/Caprate	20.00	Emollient
	Cegesoft® PS 6	Olus Oil [EU], Vegetable Oil [CTFA]	20.00	Emollient
	Cegesoft® PFO	Passiflora Incarnata Seed Oil	20.00	Emollient
	Copherol® F 1300 C	Tocopherol	0.50	Antioxidant
B	Probiolift™ BC10157	Maltodextrin, Lactobacillus	0.42	Active ingredient

Live probiotic moisturizing stick (SC-FR-22-BC-50933-03)

Phase	Ingredients	INCI	% by weight	Function
A	Eutanol® G	Octyldodecanol	13.65	Emollient
	Cutina® HR Flakes	Hydrogenated Castor Oil	4.00	Structurant
	Lanette® 22	Behenyl Alcohol	8.00	Consistency agent
	Cetiol® SB 45	Butyrospermum Parkii Butter	4.00	Emollient
	Myritol® 331	Cocoglycerides	12.00	Emollient
	Cetiol® C 5C	Coco-Caprylate/Caprate	13.00	Emollient
	Cosmedia® Gel CC	Dicaprylyl Carbonate, Stearalkonium Hectorite, Propylene Carbonate	15.00	Rheology modifier
B	Vivapur CS Sensory 15S *(JRS)*	Microcrystalline Cellulose, Cellulose Gum	15.00	Skin feel modifier
	Vivapur CS 9 FM *(JRS)*	Microcrystalline Cellulose	5.00	Skin feel modifier
C	Covi-ox® T 70 C	Tocopherol	0.30	Antioxidant
	Cetiol® Ultimate	Undecane, Tridecane	10.00	Emollient
	Probiolift™ BC10157	Maltodextrin, Lactobacillus	0.05	Active ingredient

Microbiome friendly care emulsion (SC-FR-22-BC-50945-01)

Phase	Ingredients	INCI	% by weight	Function
A	Plantaquat® NC	Cetearyl Alcohol, Lecithin, Sodium Cetearyl Sulfate, Olus Oil [EU], Cetearyl Alcohol, Lecithin, Sodium Cetearyl Sulfate, Vegetable Oil [CTFA]	3.00	Consistency agent
	Cutina® HVG	Hydrogenated Vegetable Glycerides	2.50	Consistency agent
	Cetiol® RLF	Caprylyl Caprylate/ Caprate	6.50	Emollient
	Cetiol® CC	Dicaprylyl Carbonate	4.00	Emollient
B	Water, demin.	Aqua	61.35	
	1,3-Butanediol	Butylene Glycol	10.00	Emollient
	Sodium Benzoate	Sodium Benzoate	0.25	Preservative
C	Glycerin	Glycerin	5.00	Humectant
	Verdessence™ Xanthan	Xanthan Gum	1.00	Rheology modifier
D	Dermosoft 1388 *(Evonik)*	Glycerin, Aqua, Sodium Levulinate, Sodium Anisate	1.00	Auxiliary
E	Water, demin.	Aqua	4.00	
	Postbiolift™BC10152	Maltodextrin, Lactobacillus Ferment	1.00	Active ingredient
F	Citric Acid (20% solution)	Citric Acid	0.40	pH Adjustment

Concentrated booster serum (SC-FR-22-BC-50939-02)

Phase	Ingredients	INCI	% by weight	Function
A	Cetiol® C 5C	Coco-Caprylate/Caprate	42.79	Emollient
	Cosmedia® Gel CC	Dicaprylyl Carbonate, Stearalkonium Hectorite, Propylene Carbonate	57.00	Rheology modifier
B	Probiolift™ BC10157	Maltodextrin, Lactobacillus	0.21	Active ingredient

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