First living probiotic anti-aging active ingredient with effective in vitro and vivo demonstration against placebo

240

S. Leoty-Okombi, M. Gault, L. Aversa, N. Pelletier, C. Thiel, V. André-Frei*
BASF Beauty Care Solutions, Lyon, France
sabrina.leoty-okombi@basf.com

Introduction:

Consumers understand the **need to maintain a balanced skin flora** to make our bodies inhospitable hosts to any invading pathogens, to maintain the acidic skin mantle and to contribute to skin beauty[1]. **The global probiotic skin care cosmetic product market is a fast-growing market**[2].However, real living probiotics are not widely used due to the **difficulty of demonstrating suitable stability** in diverse cosmetic formulations on top of clinical efficacy against placebo. Most of the cosmetic solutions currently proposed contains Lactobacilli, particularly *Lactobacillus plantarum* widely used in food or medical industry[3]. Regarding formulation composition, oily serum or cream with double packaging are proposed.

In order to offer novelty in the domain, **our intention was to compare at first the microbiome from young and aged panelists,** with a specific focus on the wrinkle area to particularly study the local aged-related difference. Then we **developped a skin-native living probiotic solution based on *Lactobacillus crispatus*** to help the aged skin to recover a younger microbiotic profile and a younger skin appearance. In order to demonstrate its performance, we firstly evaluated its impact on the skin metabolism and protection. Secondly, we studied its stability in different formulations to help formulators to use it broadly and finally we performed a clinical trial.

Materials & Methods:

1-Comprehensive clinical study of skin microbiota

One side of the face was dedicated to instrumental measurement followed by metabolite analysis and the other to microbial DNA study.

- 100 healthy female volunteers split into two groups according to their age and wrinkle grade:
  - 5-6 in aged cohort; aged more than 55 years old
  - 0-1 in young cohort; age between 18-35 years old
- Swabs from 3 different zones:
  - within the wrinkle hollow
  - crow's feet and undereye zone
  - cheek area adjacent to the earlobe (control)
- Whole-Genome Sequencing (WGS)

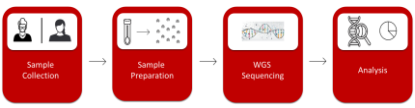

Figure 1: Skin microbiome study design

DNA was extracted and sequenced using WGS using HiSeq 3000. Taxonomic analysis was conducted using MetaPhlan2. Metabolites profiling was obtained by a combination of GC-MS and LC-MS/MS methods (Figure 1).

2-Probiotic production

The probiotic strain *Lactobacillus crispatus* (LC Probiotic) was isolated from healthy skin and identified after full genome sequencing. The strain was fermented and freeze dried after centrifugation to reach a concentration of above 10^6 colony forming units/g (cfu/g).

3-Biological activity

The LC Probiotic antiaging properties and more particularly its ability to stimulate the synthesis of collagen type I and V which decrease with age was evaluated on human fibroblast culture by DELFIA method.

4-Formulation and stability studies

Several formulating ingredients (emollients, emulsifiers and preservatives) were screened for their impact over a short period of time (1 h to 24h) on the viability of the LBC strain. Ingredients with a short-term neutrality toward the viability of the strain were then evaluated over a longer period (up to months, different temperatures). An optimized formulation was finally designed using the most favorable ingredients previously evaluated and it was checked for stability and strain viability at different temperatures.

5-Clinical trial

The clinical evaluation was performed on 29 Caucasian women (45-65 years old). The anti-aging efficacy was measured after 3 and 8 weeks. Dermis density was evaluated by ultrasound imaging with a DUB SkinScanner coupled to image analysis, and wrinkles analysis by VISIA CR imaging.

Results & Discussion:

1-Comprehensive clinical study of skin microbiota

- **Significant decrease of fatty acids and lipids for the old cohort.**
- **Alpha diversity significantly increased in the old** vs young cohort in all areas.

Surprisingly,

- **Lactic acid bacteria prevalence** in the young cohort **decreased in** the wrinkle area of **old skin.**
- Most prevalent lactic acid bacteria were Lactobacilli.
- In the **wrinkle hollow: *L. crispatus* was below** the detection level.

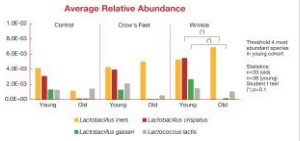

Figure 2: Abundance of lactic acid bacteria in the wrinkle of older skin.

2-Biological activity

- **Significant stimulation of collagen I** (+133%) and **collagen V** (+55%) vs untreated condition.

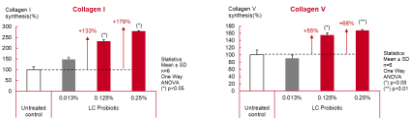

Figure 3: Collagen I and V content with LC Probiotic.

3-Formulation and stability studies

- The viability of the LC probiotic ingredient was **preserved at both 4°C and room temperature for one year** and at least 2 months at 40°C (Figure 4).

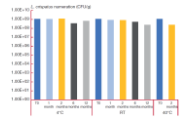

Figure 4: Stability of the probiotic ingredient.

4- Clinical trial

After 2 months of twice-daily application:

- **Significant increase** (vs baseline) of the **density of the sub-epidermal zone** by 11% and of the **total dermis** by 6%. The improvement of dermal density (+5%) was also significant vs the placebo (Figure 5).
- **Visible correction of forehead wrinkles** by -5 % vs placebo (Figure 6).

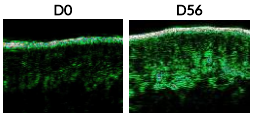

Figure 5: Illustrative pictures of the echogenic density with LC Probiotic - Volunteer 10.

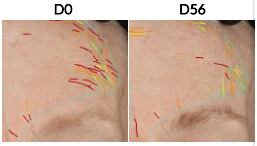

Figure 6: Illustrative picture of forehead expression wrinkles using VISIA - Volunteer 27.

Conclusions:

Unlike other probiotics existing on the cosmetic market, the newly developed ingredient is the **first to use a bacterium that is found naturally in the skin: *Lactobacillus crispatus***, a Gram-positive rod shape anaerobic bacterium that has been found to decrease with age.

However, what has become common practice in the food industry field is now one of the most **challenging tasks** for the personal care industry: incorporating living skin bacteria into cosmetic formulations and keeping them active.

Here we propose a **probiotic solution made of living but dormant *Lactobacillus crispatus* bacteria which awaken in contact with water on the skin**. This ingredient is one of the cosmetic ingredients containing skin-native live probiotic that helps make the skin feel fuller and improves the appearance of forehead wrinkles.

References:

1. *Loulou V, Panayiotidis MI. Functional role of probiotics and prebiotics on skin health and disease. Fermentation. 5,41, 2019*
2. *Puebla-Barragan S, Reid G. Probiotics in cosmetic and Personal care products: trends and challenges. Molecules. 26, 1249, 2021*
3. *Lee DE, et al. Clinical Evidence of Effects of Lactobacillus plantarum HY7714 on Skin Aging: a Randomized, Double Blind, Placebo-Controlled Study.J. Microbiol. Biotechnol. 25(12): 2160–2168, 2015*