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Meeting the challenge of formulating a live probiotic to expand its use in cosmetics

Allison Garlet[1]; BASF Corporation, Tarrytown, NY 10591, USA

Sabrina Leoty-Okombi[2]*; Corinne Thiel[2]; Valerie Andre[2]; BASF Beauty Care Solutions, Lyon, France

Introduction of research

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. 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.

Our aim was to develop a suitable formulation to deliver an efficient dose of a living Lactobacillus crispatus clinical (LC) strain previously demonstrated to be decreased in the wrinkle hollows of aged people and to prove its antiaging efficacy in vitro and in vivo against placebo. Moreover, we evaluated several components of cosmetic formulation and modes of manufacturing to propose several formulations suitable to our LC Probiotic, from serum, balm, to solid stick or even make up powder

Methods

The probiotic strain Lactobacillus crispatus (LC Probiotic) was isolated from healthy skin and identified after full genome sequencing. After fermentation in MRS medium to reach a concentration of around 10^9 colony forming units/g (cfu/g), the biomass was collected. A part of the biomass was kept alive and freeze-dried with maltodextrin (= living biomass), whereas the other part was thermally inactivated (=inactivated biomass). The lysed LC was obtained by sonicating of the suspension of LC biomass.

Collagen type I and V synthesis were evaluated in human fibroblasts at 3 concentrations (0.013, 0.125 and 0.250%) for each condition vs the control containing only the culture medium. Deposited mature collagens type I and V were measured by Delfia method after 2 days.

To develop formulas for the delivery of the probiotic, the impact of several oily ingredients for anhydrous formulas, preservative systems and specific processing steps (grinding or heating from 60 to 80°C) was screened on LC viability when stored at 4°C or room temperature (RT).

The clinical evaluation was performed on 29 Caucasian women (45-65 years old). The anti-aging efficacy was measured vs placebo after 3 and 8 weeks of twice-daily treatment by a minimum of 5 10^5 cfu/g living LC applied on the skin in a specific

formulation checked for stability up to one year at different temperatures. Dermis density was evaluated by ultrasound imaging with a DUB SkinScanner coupled to image analysis, and wrinkles analysis by VISIA CR imaging.

Results

When tested in the same condition as LC Probiotic, no collagen I and collagen V syntheses stimulation were evidenced for the inactivated or lysed biomass whereas the living biomass induced a significant increase of type I and V collagen since the 0.125% concentration (figure 1). This suggests that collagen I and V stimulation capacity is related to the fact that LC is alive.

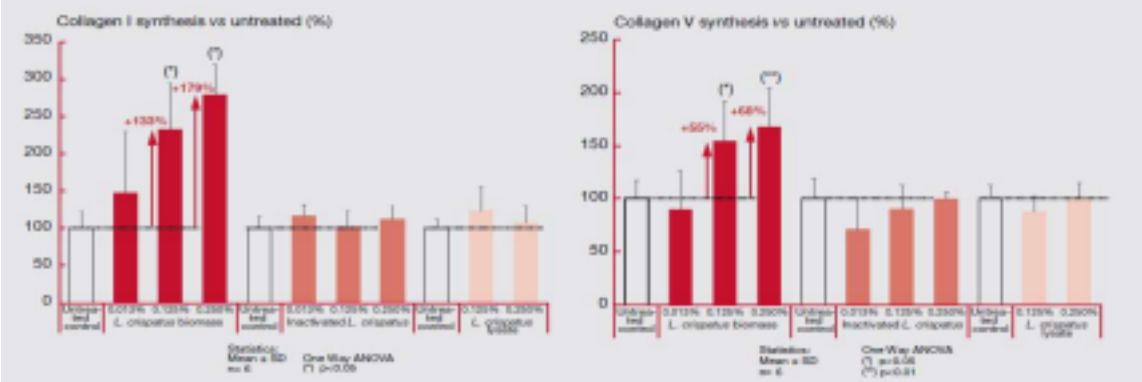

Figure 1: Collagen quantification as a function of the LC status (living, inactivated or lysed).

We first checked the viability of dried LC over one year at 4°C and room temperature, and 2 months at 40°C and did not observe any significant loss. In formulation, the probiotic strain was viable and stable in some natural oil-based emollients for several weeks, but for only up few hours in some emulsifiers or mild preservatives (figure 2).

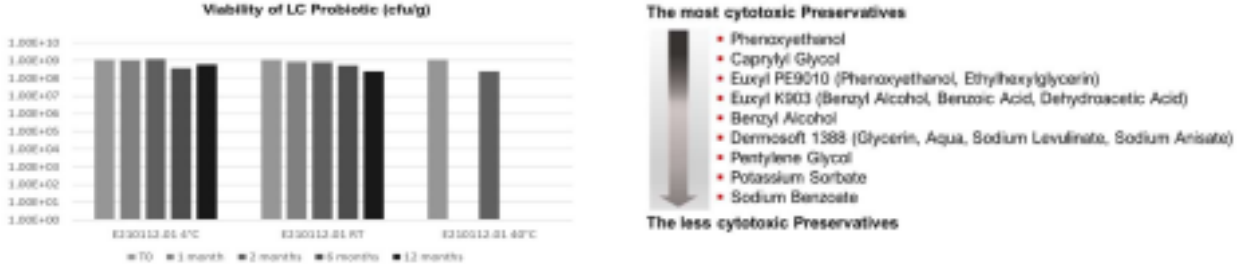

Figure 2: Viability of LC Probiotic and impact of preservatives

We succeeded in developing easy-to-use concentrated booster serums, long-term moisturization body oils with a high level of ingredients of natural origin and nomad solid stick. Stability during specific processes allowing wax use (70°C, 30mn) and grinding were also validated. The RT stability of LC in caring balm and make-up powder does not yet meet our expectations and could be still improved.

For the clinical trial, we developed an application routine where an oily serum of concentrated probiotic booster was diluted in a microbiota friendly emulsion before application to deliver the required probiotic dose. The LC strain was preserved for more than 6 months at RT in the selected emollient. This emollient was safer for LC and presents a greener impact vs mineral oil. After 2 months of twice daily application, the dose of 5 10^5 cfu/g formulated LC Probiotic induced a significant increase of the dermis density by 6% vs baseline (+5% vs placebo) and subepidermal density by 11% vs baseline. The LC Probiotic also decreased the forehead wrinkle thickness vs baseline and placebo formulation (-5%).

Conclusion

There is a rise in consumer preference for natural and organic beauty products due to the increasing adoption of a holistic and healthy lifestyle. Taking care of skin microbiota contributing to skin beauty is a trend but also a challenge when it comes to the use of live probiotics. In this study we have opened the field of possible formulations of skin native living but dormant Lactobacillus crispatus probiotic to promote its use while offering multiple galenical experiences to consumers without compromising antiaging efficacy.

About the speaker

Allison Garlet

Technical sales specialist for bio-active ingredients with 10 years of experience in early stage bioscience R&D, project management, and method development.