Synbiotic skin care with Bacillus spores

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Microorganisms populate almost every area of our planet. They are found in environments ranging from the deep sea, hot springs and other extreme environments to more apparently benign habitats closer to home, including quite literally any surface in our homes but also those of the human body. The gut microbiome is well known, but bacteria, yeasts and moulds also thrive on the surface of our respiratory tract and skin.

The skin microbiome in particular has attracted much attention from skin care professionals in recent years. It is now well established that maintaining a well-balanced equilibrium of various species is key to the skin's—and ultimately our own—health and that any imbalance in the microbiome's diversity is at the origin of multiple skin disorders.

Maintaining or improving a healthy skin microbiome the use of pre-, pro- and postbiotics is gradually gaining importance in personal care. The main challenge lies in the area of probiotics, which implies the introduction of living bacteria into a cream, lotion or other formulation.

Although several products in the market claim to contain probiotics, it very often turns out that the active ingredient is a bacterial cell lysate rather than live bacteria. Some brands, though, have successfully overcome the challenge of inoculating products without spoiling them and reaping the benefits of true probiotics. In this paper, we describe the benefits and challenges related to live probiotic strains and prebiotics in cosmetic products.

Importance of the skin's microbiome

The skin is a complex and dynamic ecosystem that is inhabited by bacteria, archaea, fungi and viruses.[1,2] These microbes—collectively referred to as the skin microbiome—are fundamental to skin physiology and immunity. Interactions between skin microbes and the host can fall anywhere along the continuum between mutualism and pathogenicity. It has been shown that there is a complex dialogue between skin microbes and the host, with consequences in terms of health and disease.

From the recent review by Callewaerts, it is clear that the skin microbiome is very different, depending on body location, but is rather stable over time.[3] Yet, many parameters can negatively influence the stability and diversity of the microbiome, including sunlight, temperature changes, pH, moisture content and even the antimicrobial or immunological responses of the skin itself.

Numerous studies have also demonstrated a link between skin health and gastrointestinal health.[4] Probiotics and changes of diet have been successfully applied to (partly) restore skin disorders,[5] and the idea of modulating the skin microbiome positively through the applications of probiotics, or even microbiome transplantations, is gaining scientific attention.

Probiotic & synbiotic skincare

Similar to the well-known use of probiotics and prebiotics to positively influence the gut microflora, probiotic and synbiotic skincare technology was recently introduced into the skincare market to modulate the skin microbiome. The best-known probiotic bacteria include members of the genera Bacillus, Lactobacillus and Bifidobacterium.

Bifidobacterium are anaerobic and are therefore not suitable for external or topical use. Lactobacillus, on the other hand, is a respected member of a good gut microflora and is often used in food applications, though it has also been used in cosmetics. Bacillus is mostly found in nature (such as soil and water) and very adapted to changing conditions (Figure 1). It is one of the most widespread species and as such also frequently found on skin.

Figure 1: Bacillus sp.
Note: Bacillus species, such as B. subtilis, are Gram-positive, rod-shaped bacteria that form dormant spores

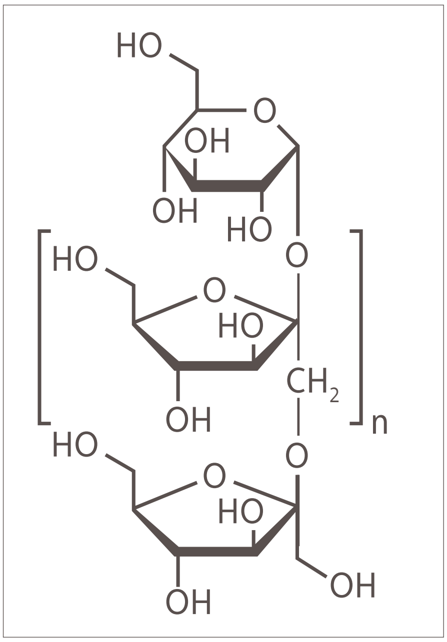

Figure 2: Inulin, an ideal prebiotic
Note: Inulin is a polydisperse mixture of linear fructose polymers and oligomers in which the fructose moieties are linked by β(2-1) bonds. A glucose molecule typically caps the end of each fructose chain. The chain lengths of these fructans range from 2 to 60 units

Because the introduction of probiotic bacteria to the skin microbiome may render the microbiome too 'artificial', prebiotic sugars, such as inulin or oligofructose, are added next to the probiotic bacteria. This will boost the performance of the probiotics in the product, and second, promote the development of already present 'desirable' organisms and as such promote microbiome diversity.

HeiQ Chrisal has developed a symbiotic, patented synbiotic concentrate containing Bacillus spores from selected species in combination with inulin (Figure 2).[6] The spores offer an elegant solution for incorporating bacteria in a cosmetic product (Figure 3). Because of their robustness towards external factors like low and high temperatures and pH, as well as good compatibility with other active ingredients, they can be easily used in cold and hot processing.

This makes them very versatile and amenable for use in almost any type of cosmetic product. Moreover, they have a much lower production cost and an extended shelf-life. Once applied on the skin the latent spores develop in living bacteria. In order to speed up this transition, inulin was added as a prebiotic booster (Figure 4). This reduces the germination time from one hour to 30 minutes. Certainly, for hand hygiene products, this booster effect helps to get fast result and protection.

Benefits of probiotic & synbiotic skincare

The initial idea of using probiotics for application on then skin was for the purpose of improving infection control. Vandini *et al.* had used Bacillus probiotics in hard surface cleaning

Figure 3: An example of a synbiotic product
Note: Ahava's probiotic line contains HeiQ Chrisal's synbiotic concentrate.

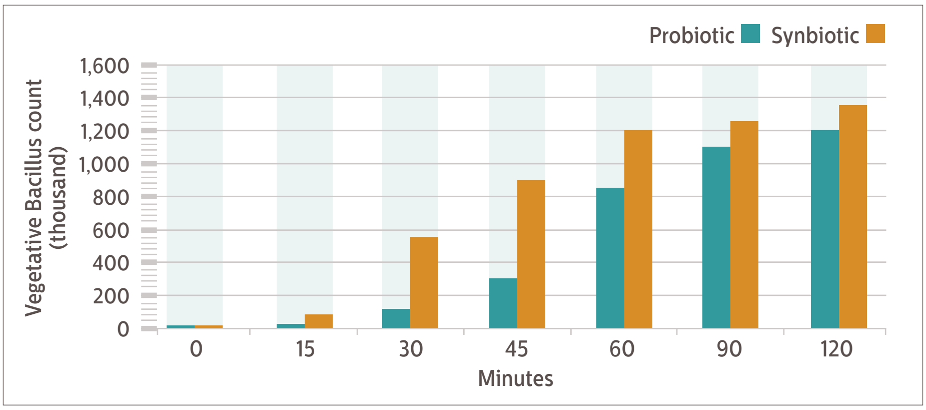

Figure 4: Prebiotic booster effect
Note: Adding inulin to the probiotic product accelerates the germination of probiotic Bacillus spores. Faster germination leads to a faster probiotic effect which is important in hand hygiene products for instance

to reduce the risk of infectious organisms, thereby greatly improving safety in hospitals.[7]

Because hand hygiene is also a major aspect of infection control, healthcare institutions asked for a probiotic hand soap or gel to be developed for use in combination with the probiotic cleaning products. This approach was very successful in reducing the number of hospital infections.[8]

The COVID-19 pandemic has also influenced the scientific focus of many academics and R&D companies. Looking at the interactions between bacteria and viruses has indicated that viruses can use some bacterial and fungal members of the skin microbiome to survive and multiply in order to facilitate their spreading.[9] On the other hand, probiotic Bacillus species such as *Bacillis subtilis*, have demonstrated to excrete the biosurfactant Surfactin, which 'degreases' the virus envelope, thereby lowering viral infectivity up to 1,000 times.[10]

More recently, research towards the mode of action and certain metabolites (postbiotics) produced by the probiotics has shown several additional health benefits related to probiotic skincare. The anti-inflammatory activity of several probiotic Bacillus species has been investigated and has been shown to be of great value for skin protection and lowering symptoms related to a number of skin disorders.

Again, the surfactins excreted by *B. subtilis* have shown to be anti-inflammatory.[11] Applying these probiotic species in skin cream may lead to less skin inflammation (redness) as a result of eczema, psoriasis or even the intensive wearing of face masks and medical gloves. Combining the infection control and anti-inflammatory benefits, a promising new application for probiotic and synbiotic skincare is wound treatment. This would lower the risk of infections by opportunistic pathogens and improve the healing process, with less risk of scar formation.

This application was first described by Savitskaya in 2019 and several clinical trials are currently ongoing to further explore probiotic wound healing.[12] These results also suggest a positive effect of probiotics on the skin barrier of intact skin, resulting in an increased hydration. However, more detailed studies on the actual mechanism and probiotic metabolites (postbiotics) involved in such recorded benefits are needed.

On the edge between skin and our inner body, probiotics have also been applied to colonise the mucosal membrane in our nasal cavity.[13] The same principle of using probiotics to modulate the upper respiratory tract microbiome has proved to be very promising in preventing several infections or even allergic reactions.

Challenges with probiotics in skincare

As with all new technologies, probiotic and synbiotic skincare products are confronted with a number of challenges, including regulatory, technical and scientific issues.

Today, Cosing refers to Bacillus and Lactobacillus species with the INCI names Bacillus, Bacillus Ferment, Lactobacillus and Lactobacillus Ferment. Although this covers almost all currently used probiotic species in cosmetics, more specific nomenclature would be welcome. Many benefits can be species- or even strain-dependent. Nevertheless, the fact that the most commonly used probiotics are already listed in Cosing is important and shows that this technology is trending.

In addition to the specific nomenclature, a common issue around most parts of the world is that cosmetics legislation is not yet fully up to date with using live microorganisms in cosmetic products. As such, certain analysis protocols for quality control (QC), as well as standard challenge tests in view of product information files, have to be expanded or modified.

Because a probiotic or synbiotic cosmetic will intentionally contain millions of probiotic micro-organisms, their presence has to be distinguished from possible contaminants. Analytical protocols aiming at total count detection will generate false positives and well-known selective detection methods have to be applied for the specific detection of contaminants.

By far the most important challenge is the stability and shelf-life of the cosmetic product containing live micro-organisms. Active probiotics should still be in full count at the end of a commercially reasonable shelf-life, which is 12 months after opening.

Because of this difficulty, today many manufacturers prefer to use heat-killed probiotics or cell extracts, although in such cases it should not be permitted to refer to the product as probiotic or synbiotic. One needs the probiotics alive to benefit from the many advantages they can offer. Those manufacturers that use live bacteria either focus on micro-encapsulated Lactobacillus species or the use of Bacillus spores.

By choosing to work with food grade probiotics, such as those listed on the Qualified Presumption of Safety list by the European Food Safety Agency, many safety concerns are already being answered. In addition, the manufacturer should pay close attention to additional safety testing and certainly establishing all relevant QC procedures that can deal with probiotic microorganisms in the product.

A good example of safety and QC criteria for products containing intentionally added micro-organisms can be found with the EU Ecolabel criteria for professional cleaning products. Respecting these criteria will make sure no safety issues will ever happen with probiotic cosmetics and the product being of good quality with an acceptable shelf life.

The final challenge is related to the production process and QC. The versatility of the HeiQ Chrisal Bacillus ferment implies that it can easily be added to the cosmetic product either during or at the end of the blending process. As mentioned above, specific QC protocols need to be in place to distinguish between the probiotic organisms and any contaminants. These will also make it possible to verify that the right amount of probiotics has been added.

Figure 5 shows the use of selective agar plates to detect contaminants among probiotic bacteria in a product. Although the probiotic Bacillus can also grow on some selective agar media (such as McConkey), their colonies do not discolour like contaminants and as such a distinction between the probiotic and a contaminant is visually possible.

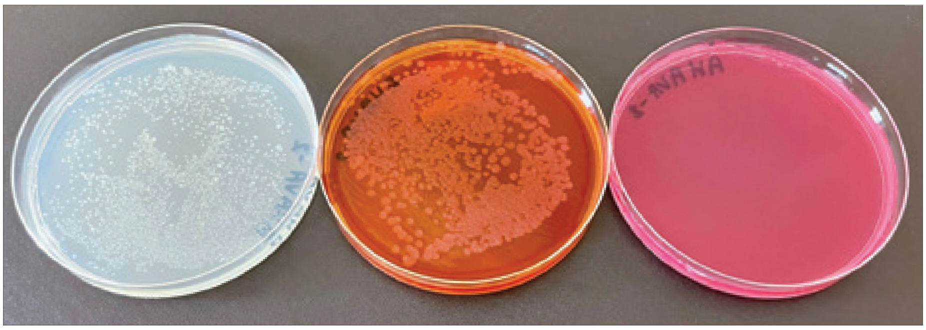

Figure 5: Modified QC procedures for probiotic cosmetics using nutrient agar for total count
Note: All colonies are the probiotic bacteria; entero count (the colonies seen are also probiotic bacteria; contaminants would have yellow/red colours) and yeast/mould count (probiotics cannot grow on this medium)

Cleaning procedures for blending and filling equipment should be revised when working with living bacteria or spores. The use of substances like hypochlorite or hydrogen peroxide as disinfectants should be validated. A careful risk analysis of the whole process and cleaning procedures should ensure a safe way of working and reduce the risk of unwanted contamination of non-probiotic products by the ferment. This will allow probiotic products to be made without the need for specific production facilities and thus major investments.

Conclusions

Probiotic and synbiotic cosmetic products represent an emerging technology that is arriving at the right moment as a good, well-balanced skin microbiome is shown to be of major importance to our overall health. Together with regulators, the industry should develop standard criteria for this emerging technology and as science will discover new and more benefits. Claim regulation also has to be specified. Although the road ahead is quite steep, this revolutionary approach to skin microbiome modulation is extremely promising and sustainable. **PC**

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