SYNERGIE SKIN
CLEAN SCIENCE

CLINICAL DATA – BIFIDA FERMENT LYSATE SUSPENDED IN MILK MATRIX NUTRIENTS (PROBIOTIC CULTURE)

Bifida Ferment Lysate suspended in milk matrix nutrients (Probiotic Culture)
This product consists of probiotic bifido cultures, suspended and disintegrated in a biologically active milk-based matrix. It supplies the skin cells with nutritive elements and allows for the activation of detoxification processes to obtain raw materials for the production of new molecules (e.g. proteins) and energy. As a result, the metabolic activity of skin cells is increased, the skin's immune system is strengthened and the skin is protected against environmental stress.

Probiotic culture is a slightly yellowish, opaque solution and clearly miscible with water. The use of alcohol in water-based formulations should not exceed 20% w/w.

Introduction:
Milk contains many nutrients that keep us healthy. The most important of these are proteins and calcium. Milk also contains phosphorus, which is used with calcium to build and maintain bones. Many other minerals and essential amino acids are present in milk as well, but in smaller amounts. Riboflavin and other vitamins, fat, and sugar (lactose) provide the energy in milk and the flavour that makes it taste good. Especially milk produced directly after birth, colostrum, has exceptional biological activity which is important for the stimulation of development processes in newborns.

Milk has a long-standing position in beauty history. Cleopatra, for example, bathed in milk to enhance her skin's youthfulness. It is not surprising to see that, since the start of this millennium, the use of milk-based active ingredients in cosmetic formulations has shown strong and continuing growth. By fractionating the different proteins and peptides from milk, active ingredients are obtained with a multitude of extremely beneficial effects on skin, whether they are anti-wrinkle and firming effects or ingredients which are specifically suitable for the care of strongly inflamed and troubled skin.

Combining the use of probiotic bacteria in a milk-based nutrient is something the food industry has been doing successfully for quite some years now. "Pro bio" means "for life," and the probiotic bacteria, consumed orally, are thought to be maintained in the gut, binding to intestinal epithelial cells and preventing the growth of pathogenic bacteria, thus promoting gastrointestinal health. Additionally, probiotic bacteria have shown to possess many beneficial effects on immune function. In the intestines, they do so by stimulating the intestinal epithelial cells – not like a whole microbe, but with their structural components and metabolites. This has been reported to lead to an improvement of the barrier function of these cells, among other effects.

Bifidobacterium is one of the most important probiotic bacteria used in the dairy industry. It is a genus of Gram-positive, non-motile, rod-shaped anaerobic bacteria. It was found that bifidobacteria have a positive effect on the immune system and help to control the intestinal pH.

In adult intestines, only 3-6% of the microflora is composed of bifidobacteria, while in breastfed infants' bifidobacteria can constitute up to 90%. With increasing age, the number of bifidobacteria decreases. It was observed that babies and adults with lower numbers of bifidobacteria have a higher risk of diarrhea and allergies. This is why bifidobacteria are added as a probiotic supplement to infant formulas, drinks, and yogurts.

In recent years, evidence has emerged that the oral consumption of probiotic bacteria can have a positive effect on the skin. It was shown, for instance, that the skin's resistance against UV radiation was strengthened, leading to better preservation of skin homeostasis. Oral probiotics were also shown to be supportive in the treatment of atopic dermatitis. Key in this activity is the fact that oral probiotics can enhance the presence of regulatory T cells in the skin, effectively reducing the inflammation. A similar mechanism induced by oral supplementation of probiotic bacteria was observed in the treatment of eczema. These beneficial effects on the skin are presumed to be initiated in the gut.

Another target for probiotics was shown to be the skin barrier function. A 24-week skin nutrition with a fermented dairy product in female volunteers with dry and sensitive, but otherwise healthy skin significantly reduced transepidermal water loss, thus improving the stratum corneum barrier function compared to a placebo product.

Applying probiotic bacteria topically might be beneficial for the skin for cosmetic purposes, but is not feasible, as introducing and managing live bacteria in cosmetic formulations is difficult. Moreover, the harsh environmental conditions on the skin prevent the colonisation of skin with probiotic bacteria. As mentioned above, however, it is the structural elements and metabolites which are essential in obtaining biological benefits from probiotic bacteria. In recent literature, it was reported that the topical application of a lysate of probiotic bacteria acts on the skin by specifically interacting with toll-like receptors (TLRs). TLRs are present on the outer membrane of keratinocytes and play an active part in the immune system. They are able to recognise specific molecular patterns, which leads to the stimulation of the immune system when lysates of probiotic bacteria are applied topically. Cosmetic active ingredients on the basis of lysates of probiotic bacteria have shown to be powerful active ingredients for skincare, especially when combined with milk proteins, essentially mimicking probiotic drinks.

Activity

Probiotic culture consists of probiotic bifidobacteria disintegrated in a bioactive milk-based nutrient. It constitutes a complete approach toward probiotic nutrition for the skin. Nutritional cosmetics are a large and strongly growing segment of the personal care market. Consumers understand the need of "feeding" the skin with beneficial ingredients from cosmetic skincare formulations. Probiotic culture has multiple benefits for skin nutrition. It provides the skin and the skin cells with essential nutrients from milk, which are endocytized, after which the so-called endosomes fuse with lysosomes, enabling the digestion of the nutrients (Fig 1). Yet it also contains another, revolutionary approach toward skin nutrition.

In essence, especially in aged skin, the skin cells accumulate high amounts of defective and damaged cellular components. The accumulation of this cellular "waste" is considered one of the hallmarks of ageing. Interestingly, Probiotic culture is able to activate intracellular systems, allowing for the disposal and recycling of cellular waste, which essentially means that it can activate cellular detoxification systems and enable the reuse of the waste. The endpoint of the activation of these so-called autophagy processes, during which cellular waste is "engulfed" by autophagosomes, is similar to that of providing the cells with essential nutrients from milk – the cells become healthier and more vital (Fig. 1).

This dual approach to cellular nutrition with Probiotic culture illustrates its potency as a cosmetic active ingredient for skincare formulations, but not just for nutritional cosmetics. It additionally strengthens the immune system, for instance, as would be expected from a product based on probiotic bacteria. This activity spectrum of Probiotic culture was proven *in vitro* on skin cells and *in vivo* on human skin.

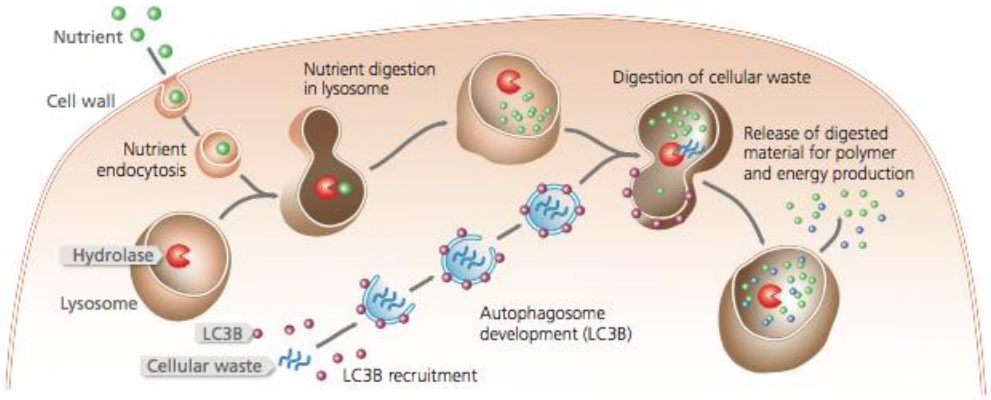

Figure 1 - Intracellular processes toward nutrient digestion and autophagy of cellular waste.

Efficacy studies
In vitro assays (cell culture tests)

Maintenance of a balanced immune system.
Interleukins are cytokines that act as signal molecules of the immune system. These mediators are essential for the induction and complete process of immune reactions.

Immunosuppression signifies the suppression of immune responses. Immunosuppressive action can be triggered by stress of any kind, especially excessive UV irradiation of the skin.

An immunological imbalance in the skin after UV exposure has important implications not only for the exacerbation of infectious diseases but also for the generation of skin cancer. UV-induced modulation of immunoregulatory cytokines with upregulation of interleukin 10 (IL-10) and IL-12 downregulation are the critical events responsible for immunosuppression. Consequently, it is necessary to have a balance proportion

between IL-12 and IL-10 in order to avoid a down-regulated immune system due to IL-10.

Keratinocytes and immunocompetent cells release interleukin 10 (IL-10), the mediator responsible for immunosuppression. Keratinocytes pretreated with probiotic culture show reduced IL-10 expression after UV irradiation (Fig 2. and Fig 3). The active thus counteracts imbalances of the immune system.

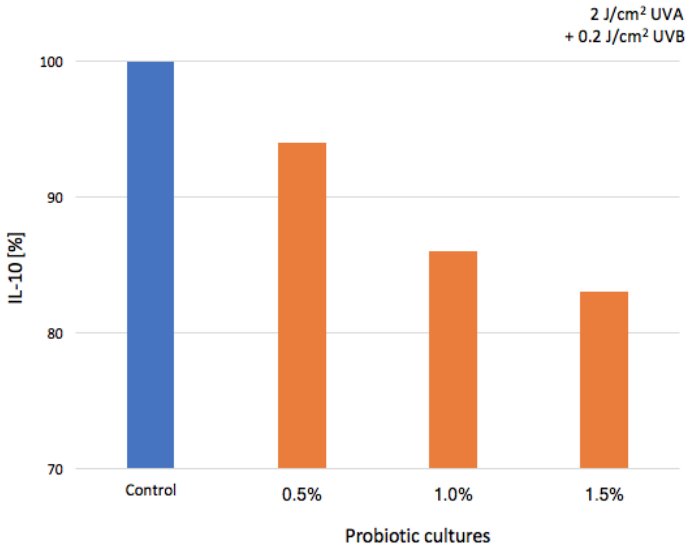

Figure 2 Influence of IL-10 immunosuppressor

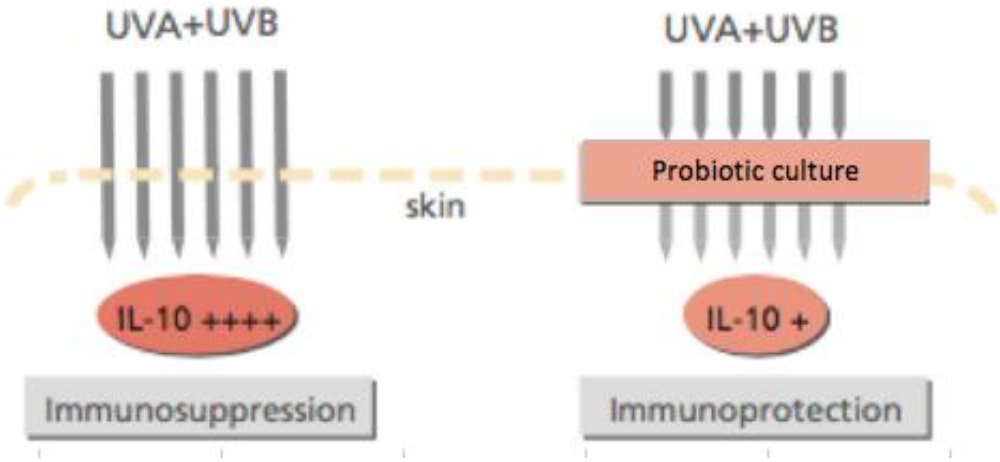

Figure 3 Negative influence of UV light on the immune system IL-10 immunosuppressor

Protection of the extracellular matrix
Influence on Cathepsin B

Cathepsins are intracellular proteolytic enzymes involved in the final catabolism of predigested proteins (for example, by matrix-metalloproteases, or MMPs). Cathepsin B represents a marker of cell damage after UV exposure (Fig. 4). UV-irradiated keratinocytes, pre-treated with Probiotic culture, showed reduced Cathepsin B activity

(Fig. 5). Probiotic culture counteracts the UV-induced degeneration of collagen and elastin, thus maintain homeostasis of the extracellular matrix composition.

Supply with nutritive elements
Lysosomal activity after undernourishment
Lysosomes are the digestive system of our cells. A battery of lysosomal enzymes digest food ingredients, macromolecules and cellular debris. The more lysosomes are active, the better the cell is nurtured.

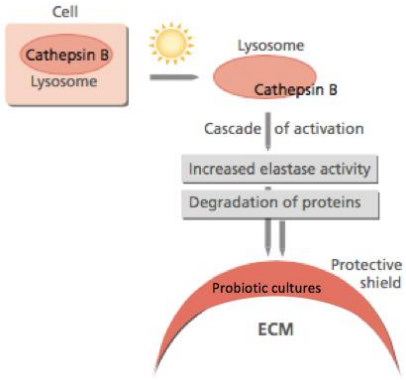

Figure 4 Protection of the extracellular matrix (ECM)

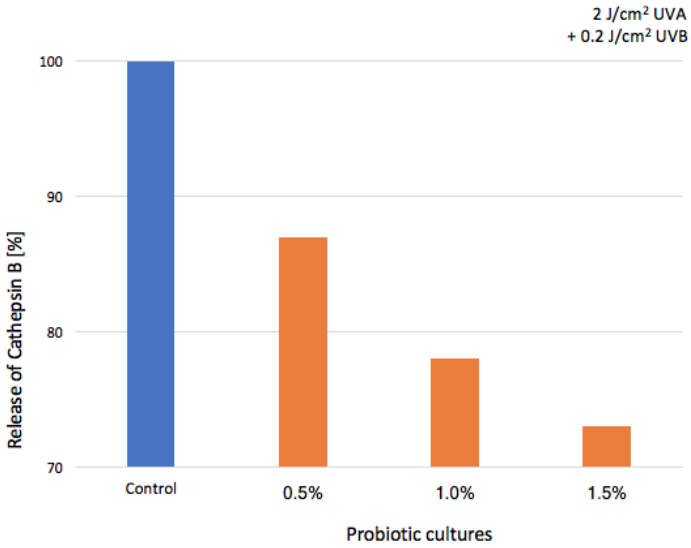

Figure 5 Release of Cathepsin B by keratinocytes after UV radiation

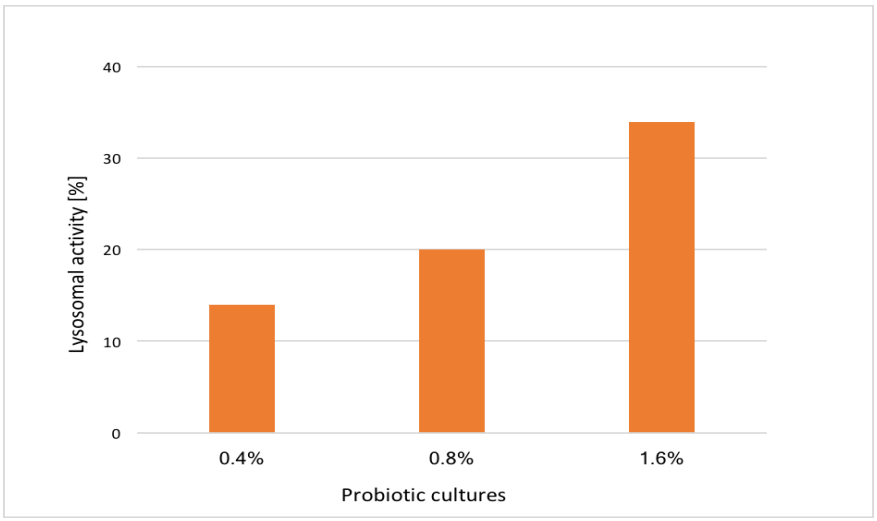

Figure 6 Increase of lysosomal activity after undernourishment

Keratinocytes were grown in a complete medium providing optimum nutrition with and without Probiotic culture. After 72 hours, the complete medium as well as probiotic culture were removed, and the concentration of nutritive substances was reduced to undernourish the cells. Under the conditions produced by the incomplete medium, the cells pretreated with probiotic culture remained on a higher vitality level and could cope with stress situations without difficulties (Fig 6. & Fig 7).

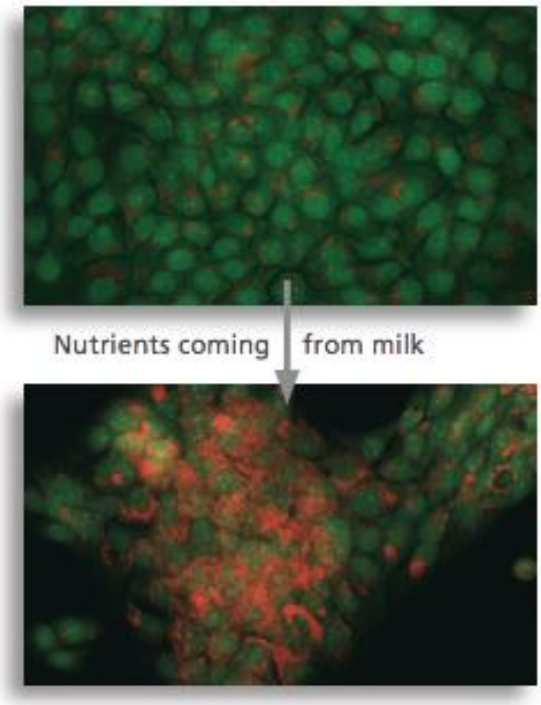

Figure 7 Supply with nutritive elements

Activation of cellular waste disposal and recycling

Cellular waste can be disposed of and made available for reuse by autophagy. During this process, cellular waste is captured in autophagosomes. The autophagosomes then

fuse with lysosomes, allowing for the digestion of the cellular waste. The outer cell membrane of autophagosomes contains a unique protein called LC3B, which serves as a general marker for autophagic membranes and for monitoring autophagosomes production as they develop.

Cells were grown in the presence of Probiotic culture. At different concentrations, Probiotic culture shows an increase in LC3B (Fig 8.), leading to the conclusion that it induces autophagosome production and, therefore, cellular waste disposal and reuse.

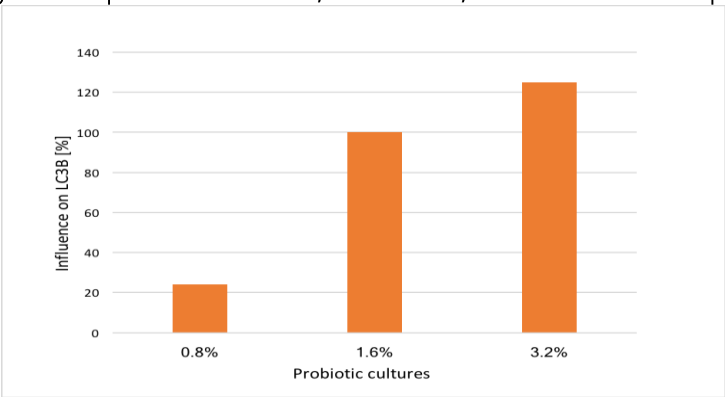

Figure 8 Increase in autophagosome production and cellular waste disposal and reuse.

Increase in metabolic activity

A seemingly logical consequence of cellular nutrition – whether by supplying nutritive elements or activating cellular waste disposal and reuse - is that the skin cells become more vital and show higher levels of activity. This is indeed the case with Probiotic culture. In an MTT assay, a colorimetric assay where the activity of NAD(P)H-dependent oxidoreductase enzymes is quantified, the metabolic activity of cells, which allows them to grow and reproduce, was determined. The metabolic activity of cells was increased depending on the concentration of Probiotic culture (Fig 9.)

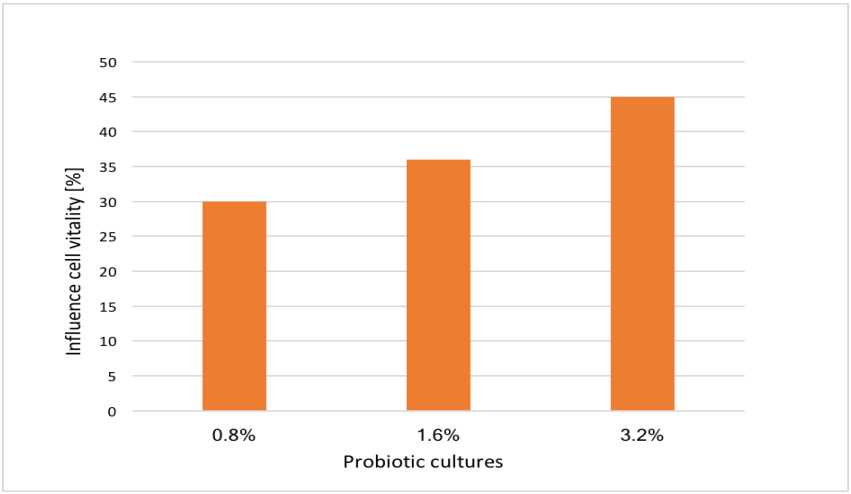

Figure 9 Increase in cellular metabolic activity

Efficacy Studies

In vivo assays (human volunteers)

Day after day, our whole body is exposed to both environmental stress and the strains of everyday life. Under stress conditions, we are more short-tempered, our immune system deteriorates, and our skin becomes more sensitive, easily irritated and thus vulnerable. Probiotic culture act as a protective shield for the skin. It helps reduce irritability of the skin and at the same time protects it from additional stress. The less the skin is irritated, the more balanced it is.

Probiotic cultures lead to a more balanced skin, as could be demonstrated *in vivo* in 20 volunteers: Defined skin areas were treated with SDS to induce redness (irritation). Decrease in redness was then recorded for skin areas treated twice daily with a formulation containing 5% Probiotic culture and compared with placebo and untreated controls. On Day 9 the SDS treatment was repeated and the development of skin redness recorded.

An O/W cream with 5% Probiotic culture was shown to reduce the visible signs of skin irritation, and the skin was much better protected against skin damage compared to untreated and placebo (Fig 10.).

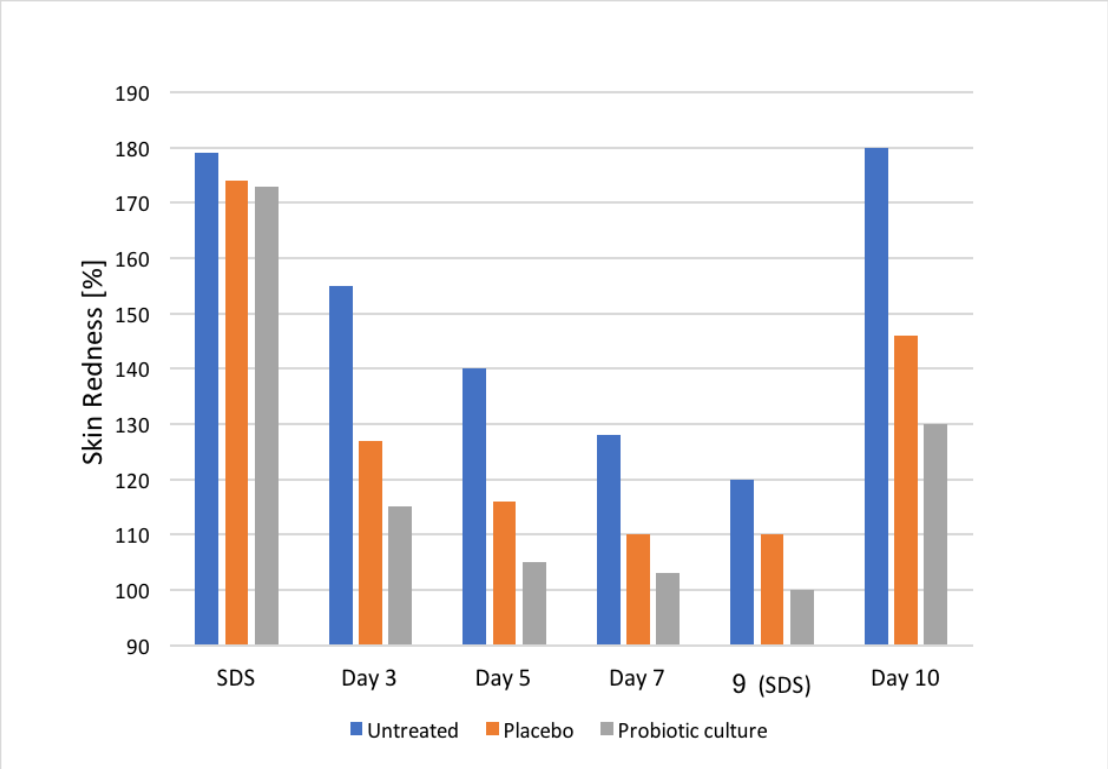

Figure 10 Reduction of skin irritation and protection against skin damage

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