Use of Probiotics for Dermal Applications

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Abstract The concept of probiotic bacteria is considerably evolving. Clinical and experimental researches extensively document that beyond probiotic capacity to influence positively the intestinal functions, they can exert their benefits at the skin level thanks to their peculiar properties. Indeed, scientific and evidence-based reports strengthen the assumption that certain probiotics can contribute to modulate cutaneous microflora, lipid barrier, and skin immune system, leading to the preservation of the skin homeostasis. In this chapter, the most relevant evidences available from scientific literature as well as registered patents have been summarized in relation to actual or potential topical applications of probiotics in the field of dermatology. Altogether the evidences reported in this review afford the possibility of designing new strategies based on a topical approach for the prevention and treatment of cutaneous disorders.

1 Introduction

Most often in foods and oral formulations, the probiotics are mainly used as a means for restoring microbial balance, particularly in the gastrointestinal tract (Williams 2010). This approach appears particularly significant since the intestinal microbiota is involved in physiological balance and in the intestinal development and maturation of the host immune system (Oelschlaeger 2010). Thus, in the last years an increasing interest has been focused on the possible use of ingested probiotics for treating inflammatory and allergic conditions being specific strains able to modulate the immune system at the local and systemic levels (Caramia et al. 2008). Arck et al. (2010) have recently hypothesized a new, unifying model i.e., gut–brain–skin axis, suggesting that modulation of the microbiome by deployment of probiotics can exert profound beneficial effects on skin homeostasis, skin inflammation, hair growth, and peripheral tissue responses to stress. On the other hand, new insights could now fundamentally change the impact of probiotics on dermatology. Indeed, an emerging approach to help preventing and treating skin conditions, including the external signs of aging, acne, rosacea, yeast and bacterial infections, psoriasis, and dermatitis, is represented by topical probiotics, as shown by the growing marketplace for topical probiotic formulations available for skin care and antiaging benefits. In this chapter, the key evidences available from scientific literature as well as registered patents will be summarized in relation to actual or potential topical applications of probiotics in the field of dermatology.

2 Probiotics and Skin Microflora

The skin is able to act as a physical barrier exerting several functions such as fluid homeostasis, thermoregulation, immune responses, neurosensory functions, metabolic functions, and primary protection against infection. The skin microflora plays a significant role in competitive exclusion of pathogens that are aggressive and provoke infection in the skin and in the processing of skin proteins, free fatty acids (FFAs), and sebum. Interestingly the resident microbiota may be regarded as "beneficial" to the normal, healthy host, but may become dangerous to the host with disturbed skin integrity. Microorganisms may have a role even in atopic dermatitis (AD), eczema, rosacea, psoriasis, and acne. Even if there are only very few studies pursuing on probiotic approach for microflora-related skin disorders, it is intriguing to suppose that topical probiotic application can be beneficial either for preventing or for treating altered microflora-associated skin diseases (Krutmann 2009; Simmering and Breves 2009).

2.1 Production of Acids by Probiotics

Preservation of the resident microflora is thought to be an effective way to achieve maintenance of healthy normal skin functions; however, the number of microbial species colonizing human skin is limited depending on the hard physical and biochemical factors. Probiotic microorganisms use different mechanisms, such as by lowering pH, to preserve skin health and to inhibit the growth of pathogens. The acidic skin environment is indeed very important as it discourages bacterial colonization and provides a moisture barrier through absorption or moisture by aminoacids, salts, and other substances in the acid mantle (Lambers et al. 2006; Mauro 2006). An interesting property of probiotics is the fermentative metabolism that involves the production of acid molecules (i.e., lactic acid), thus acidifying the surrounding environment (Krutmann 2009).

2.2 Antimicrobial Substances

The potential topical use of probiotic strains capable of producing potent antimicrobial toxins (i.e., bacteriocins, bacteriocin-like substances, organic acids, and H_2O_2) has received increasing attention to successfully prevent pathogen adhesion and outcompete undesired species (Oh et al. 2006; Gillor et al. 2008). Topical compositions containing probiotic bacteria, spores, and extracellular products and uses thereof represented the basis of the invention of Farmer (2005) suitable for topical application to the skin which can be utilized to inhibit the growth of bacteria, yeasts, fungi, viruses, and combinations thereof. The invention also

disclosed methods of treatment and therapeutic systems for inhibiting the growth of pathogens and combinations thereof, by topical application of therapeutic compositions which were comprised, in part, of isolated *Bacillus* species, spores, or an extracellular product of *Bacillus coagulans* comprising a supernatant or filtrate of a culture of *Bacillus coagulans* strain. A method and composition were also provided for application of probiotic microorganisms to a surface to prevent or inhibit contamination by pathogenic microorganisms (Spigelman and Ross 2008). The probiotic microorganisms may be bacteria, yeast, or mold. Suitable probiotics should be selected according to one or more particular properties, being the preferred properties of their competitive exclusion of pathogenic organisms from the surface to which they are applied, adherence to human tissue, sensitivity to antibiotics, antimicrobial activity, acid tolerance, and a high oxygen tolerance. In particular, the method consists of different application modalities (i.e., lotions, spraying, wipe paper) of one or more probiotic microorganisms to a wide variety of surfaces, such as human skin and hospital equipment and fixtures, in an amount effective to, at least partly, prevent their contamination, colonization, growth, and cross-contamination by the pathogenic bacteria. The method relies on the probiotic ability to form isolated colonies producing a protective layer that can inhibit and exclude pathogenic bacteria generally unable to grow on top of other bacteria. The probiotic application is recommended for a sufficient time depending upon such factors as the therapeutically effective amount, the type, the mode of probiotic application, or the degree of contamination of the biological or nonbiological surface. Accordingly, the method propones the use of a single or a plurality of different probiotic microorganisms, applying multiple bacteria serially, in layers to fight several or single resistant types of pathogenic organisms.

In regards to the potential use of bacteriocin-producing strains as probiotic and bioprotective agents, a number of bacteriocins produced by various lactic acid bacteria species including *Lactobacillus, Lactococcus, Pediococcus, Propionibacterium, Leuconostoc, and Carnobacterium* have been reported (Klaenhammer 1993; Oh et al. 2006). Of interest, Oh et al. (2006) reported the efficacy of the bacteriocin from *Lactococcus* sp. HY 449 in controlling skin-inflammation and acnes by clinical skin irritation test. This study demonstrated that this bacteriocin was able to inhibit the growth of skin inflammatory bacteria such as *Staphylococcus epidermidis Staphylococcus aureus, Streptococcus pyogenes,* and *Propionibacterium acnes*. The experimental data highlighted that the inhibitory effect of the bacteriocin employed in this study was due to bacteriolytic action on the cell wall and cell membranes of *P. acnes*. Thanks to its antimicrobic properties, *Lactococcus* bacteriocin could be used in cosmetic application for a variety of purposes.

The invention of Teodorescu (1999) disclosed the use of eubiotic product, consisting of a mixture of three *Lactobacillus acidophilus* strains, LD-11, LR-13, and LV-17, associated in equal parts, for the maintenance and treatment of tegument. In comparison with the common strains, LD-11, LR-13, and LV-17 can also ferment raffinose, trehalose, and dextrin, respectively. The most important aspect of this finding lies on an optimum association of three *L. acidophilus* anallergical

strains for realizing a natural eubiotic product, capable of maintaining the skin pH at physiological values, to destroy the pathogenic microflora and to be resistant in cosmetic composition.

2.3 Beta Defensins

Sullivan et al. (2009) claimed that extracts of *Lactobacillus* could stimulate the production of beta-defensins in skin cells, which may be useful in the reduction or prevention of growth of microbial populations on the skin, in a dose-dependent manner. Effective amounts of *Lactobacillus* extracts are applied to an open cut or wound on the skin that may have been in contact with dirt or undesirable microbes; or on a chronic basis, applied to clean skin to maintain a healthy level of skin flora. According to the authors, the extracts could also be useful in the treatment of acne. Indeed, topical compositions containing *L. plantarum* extract are shown to reduce the incidence of both inflamed and noninflamed acne lesions when used regularly over a period of 2 months. The extracts had further been proposed as a preservative in cosmetic of pharmaceutical products, in particular the *L. plantarum,* which possesses a broad spectrum of activity against both Gram-positive and Gram-negative bacteria. *Acne vulgaris* is multifactorial condition and is characterized by hypercolonization with *Propionibacterium acnes*, inflammation, and immune responses. The synbiotic ability of probiotic bacteria and Konjac glucomannan hydrolysates to inhibit the growth of *Propionibacterium acnes* in an in vivo study has been recently reported suggesting that the development of a new alternative involving probiotic therapy for reducing acne episodes in vivo could be encouraging (Al-Ghazzewi and Tester 2009).

2.4 Probiotics and Wound Protection

Chronic wounds are, by definition, the ones that remain in a chronic inflammatory state and therefore fail to follow the normal patterns of the healing process; factors that may impede healing must be identified and, if possible, corrected, for healing to occur. Chronic wounds, and burns in particular, are rarely, if ever, sterile. The burn wound surface is sterile immediately following injury, although it is repopulated within 48 h with Gram-positive organisms from hair follicles, skin appendages, and environment; successively, more virulent Gram-negative organisms replace the Gram-positive organisms after 5–7 days. Burns produce disruption of the mechanical integrity of the skin and generalized immune suppression that allows microorganisms to multiply freely. Currently, the common pathogens isolated from burn are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, and various coliforms. Other streptococci, anaerobic organisms, and fungi can also cause infections. Bacterial infections are generally treated by

administration of antibiotics; however, this is not always efficacious. Contributing to the lack of successful antibiotic treatment is the ability of colonizing bacteria to establish themselves and proliferate in a biofilm, a characteristic architecture of microcolonies embedded in a self-made matrix of biopolymers that offers structural stability and protection. In this composite state, the bacteria resist the action of a variety of antimicrobial measures, and moreover they are extremely resistant to antibiotics, antiseptics, and to the host immune response, so new therapeutic modalities may be required. An effective approach to prevent or contrast infection could be bacteriotherapy, i.e., the use of probiotics to displace pathogenic organisms. Valdèz et al. (2005) suggested that *L. plantarum* and/or its products are potential therapeutic agents in the local treatment of *P. aeruginosa* burn infections. The authors showed that the in vitro treatment with *L. plantarum* was able to inhibit the production of the *P. aeruginosa* quorum-sensing signal molecules, acyl-homoserine-lactones, and two virulence factors controlled by these signal molecules elastase and biofilm. On the other hand, the subcutaneous injection of *L. plantarum* on a burned-mouse model with *P. aeruginosa* burn infection, induced inhibition of *P. aeruginosa* colonization as observed in skin, liver, and spleen samples taken after 5, 10, and 15 days upon infection, thus leading to a significant improvement in tissue repair. On the basis of these encouraging findings, a preliminary study (Peral et al. 2009a) was carried out to determinate the effect of topical *L. plantarum* treatment on infected and non-infected second-degree burn patients and on infected third-degree burn patients. As a result, in non-infected third-degree burns, the ability of *L. plantarum* to prevent infection, to promote granulation tissue, and to heal wounds was comparable to the silver sulphadiazine cream one; in infected second-degree burns, *L. plantarum* treatment was as effective as the silver sulphadiazine one with reference to decrease in the bacterial load, promotion of the appearance of granulation tissue, and wound healing. In infected third-degree burns, treatment with *L. plantarum* would show great efficacy. A further study of the same group showed the efficacy of *L. plantarum* bacteriotherapy on the chronic infected leg ulcers of diabetic and nondiabetic patients (Peral et al. 2009b).

The antimicrobial efficacy of nitric oxide (NO) is well known. Of note, a recent report (Jones et al. 2010) showed that the NO-producing probiotic patch device containing lyophilized alginate-immobilized *L. fermentum*, glucose, and nitrite salts can produce sufficient levels of gaseous NO over a therapeutically relevant duration, to kill common bacterial and fungal pathogens existed in the wounds of humans.

A recent invention of Hansen and Jespersen (2010) was directed to a wound or tissue dressing comprising bacteria having the property of producing lactic acid by fermentation of the sugars, to use in healing wounds or in accelerating the wound healing. Particularly, preferred species of lactic acid bacteria including *L. sporogenes, L. acidophilus, L. plantarum, L. casei, L. brevis, L. delbruckii*, and *L. lactis* are present in the dressing, capable of (1) lowering the pH in an open wound environment, (2) securing an intraspecies competitive exclusion thus preventing growth of undesirable bacterial species, (3) exerting an

immunomodulatory effect by inducing "wound healing-promoting substances" (i.e., cytokines, growth factors), and (4) producing certain bacteriocins such as toxins that can sustain a wound-healing process.

Actual management of chronic wound and burn patients is enormously expensive. Moreover, traditional treatments and caring are often ineffective and fail to eradicate bacteria, especially in biofilm form. Bacteriotherapy with specific probiotic strains could represent a new therapeutic modality thanks to their efficacy, innocuousness, easy access to application, and low costs, even if this issue deserves further investigation for possible use in topical wound treatments.

3 Probiotics and Disturbed Skin Barrier

Cutaneous pH plays an important role in the barrier function; in particular, human normal skin pH is acidic and varies from an acidic pH of 3.0 to an almost neutral pH of 6.5. Acidic skin surface has been attributed to microbial factors, esocrine gland presence, generation of urocanic acid by histidase-catalyzed deimination of histidin, secretory phospholipase A_2-mediated generation of FFAs from phospholipids, and a nonenergy-dependent sodium–proton exchanger (Feingold 2007; Cinque et al. 2010). Cutaneous pH can control bacterial populations on skin surface affecting resident microbiota and can regulate epidermal permeability barrier homeostasis and stratum corneum (SC) integrity (Feingold 2007; Cinque et al. 2010).

3.1 Cutaneous pH

The two key cutaneous lipid-processing enzymes, β-glucocerebrosidase and acidic sphingomyelinase (aSMase), which generate a family of ceramides from glucosylceramide and sphingomyelin (SM), respectively, exhibit low pH optima (Feingold 2007; Cinque et al. 2010). An acidic pH directly impacts lipid–lipid interactions in the SC extracellular lamellar bilayers confirming the link between SC pH and barrier homeostasis and SC integrity/cohesion. Elevations of SC pH are accompanied by perturbed cutaneous permeability barrier homeostasis, and by an increase in serine protease activity that mediate degradation of corneodesmosome, resulting in abnormality of SC integrity/cohesion. In conclusion, an acute increase in SC pH reduces the activity of certain key lipid-processing enzymes in the SC, resulting in abnormal lipid processing and the formation of defective lamellar membranes. Moreover, an elevation in SC pH is associated with several cutaneous disorders, such as acute eczema, atopic dermatitis, and seborrheic dermatitis. In these diseases the increased pH could adversely affect cutaneous functions exacerbating these conditions further with more severe clinical manifestations (Feingold 2007; Cinque et al. 2010). Thus, in order to restore the altered cutaneous

functions because of increased pH, alternative strategies could be pursued in order to modulate SC pH. As stated above, an interesting approach could envisage the use of probiotic as lactic acid bacteria with fermentative metabolism that are able to produce lactic acid and obtain energy from the fermentation of lactose, glucose, and other sugars to lactate via homofermentative metabolism (Teodorescu 1999; Farmer 2005; Chiba 2007)

Of interest, Yadav et al. (2007) reported that the lipolysis of milk fat by probiotic lactobacilli increases the production of FFAs and produces conjugated linoleic acid by using internal linoleic acid. This acid-producing mechanism inhibits growth of other organisms and favors the growth of lactobacilli that thrive in low pH environments conferring a further health benefit to the host.

3.2 Ceramides

The SC lipids are secreted from keratinocytes in lamellar bodies containing lipid precursors (i.e., glucosylceramides, phospholipids, and cholesterol sulfate) and enzymes (β-glucocerebrosidase, acidic SMase, secretory phospholipase A_2, and steroid sulfatase) that generate ceramides, FFAs, and cholesterol. Within the epidermal membrane structure, the ceramides are the dominant lipid class by weight (~50%) and play an essential role in maintaining and structuring the lipid barrier of the skin. Therefore, a decrease of ceramide in SC will cause water loss and barrier dysfunction in the epidermis, including a loss of protection against antigens, including bacterial, and can result in a skin abnormality such as AD (Feingold 2007; Mizutani et al. 2009; Cinque et al. 2010). The rate-limiting enzyme for the synthesis of ceramides is serine palmitoyl-CoA transferase since its inhibition leads to delayed barrier repair. Ceramides result also from hydrolysis of cerebrosides and SM through β-glucocerebrosidase and SMase, respectively. The SC ceramide levels are thus regulated by the balance among these ceramide-generating enzymes and the degradative-enzyme ceramidase. Both SM and SMase are present in the epidermis and are originally contained in lamellar bodies; structure contains a mixture of ceramides, cholesterol, and FFA. They play a role in the formation of the lipid component of the skin barrier and in the maintenance of the SC stability. To effectively maintain the permeability barrier homeostasis, all three main lipidic components of the stratum corneum, cholesterol, FFA, and ceramides must be present. The absence or decrease of even one of these components in perturbed skin can delay barrier recovery (Feingold 2007; Mizutani et al. 2009; Cinque et al. 2010). A previous study of our group (Di Marzio et al. 1999) reported that high levels of neutral SMase was detected in sonicated *Streptococcus salivarium* ssp. *thermophilus* which was able to induce generation of relevant ceramide levels in keratinocytes in vitro. Both hydroxyceramide and nonhydroxyceramide levels strongly and gradually increased in the presence of sonicated *S. thermophilus* in a time-dependent manner due to SM hydrolysis in keratinocytes which in turn could be correlated with the high levels of a neutral SMase activity of *S. thermophilus*.

These results were confirmed by in vivo studies in healthy and young volunteers. A relevant increase in SC ceramide levels was indeed observed in all the analyzed subjects after topical application of experimental cream containing lysed *S. thermophilus* (Di Marzio et al. 1999). The presence of high levels of neutral SMase activity in the volunteers was responsible for the observed increase of SC ceramide levels, thus leading to an improvement in barrier function and maintenance of SC flexibility. The use of SMase obtained from selected strains of lactic acid bacteria to increase the levels of skin ceramides, and dermatological and cosmetic compositions suitable for topical application containing same, represented the basis of the invention of Cavaliere and De Simone (2001). According to this patent the bacterial SMase could also be used as a cutaneous permeation or absorption enhancer, either alone or in admixture with other enhancers, for preparing pharmaceuticals or cosmetic compositions suitable for transdermal administration. Thus, a method claimed to prevent or treat all conditions associated with abnormal ceramide levels (deficiencies), including skin aging, atopic eczema, dermatosis or dermatitis, atopic dermatitis, psoriasis, ichtyosis, Fabry's disease, Gaucher's disease, Tay-Sachs disease, or Sjogren-Larsson's syndrome comprising topical applying on the affected skin with a pharmaceutical composition which contain neutral SMase obtained from sonicated lactic acid bacteria. To test the possibility that the use of the *S. thermophilus*-containing cream could improve SM dysmetabolism in AD patients, our group previously conducted a study to determine the effect of sonicated *S. thermophilus* on the level of ceramides in vivo in the skin of AD patients (Di Marzio et al. 2003). A 2-week application of the cream containing a sonicated *S. thermophilus* in the forearm skin of the patients led to a significant and relevant increase of skin ceramide amounts, which could result from the SM hydrolysis through the bacterial SMase. Of note, the topical treatment consequently led to a reduction of the AD-associated signs and symptoms such as erythema, scaling, and pruritus in all patients.

Some reports in the last decade have described the changes in total SC with increasing age, in particular the reduced corneum lipid levels (Cinque et al. 2010). Indeed, all major lipid species in the stratum corneum of aged human and mice skin decreased by approximately 30%. In addition, the neutralization of the normally acidic SC has deleterious effect in permeability barrier homeostasis and SC integrity/cohesion. Considering the role of ceramides in SC, our group investigated the short-term topical application of a probiotic formulation on healthy skin of old Caucasian women (Di Marzio et al. 2008). Increase in skin ceramide levels in aged subjects following a short-term topical application of bacterial SMase from *S. thermophilus* has been reported. Consequently, the skin hydration and ceramide levels as markers of functional skin were determined. The results of this study showed hydration effects increase in the skin of subjects treated with *S. thermophilus*-containing cream when compared with controls. The hydration skin increase could be attributed to the enhanced SC ceramide levels probably due to the SMase presence in *S. thermophilus*. These findings suggested that topical application of a sonicated *S. thermophilus* preparation may contribute

to the improvement of lipid barrier and a more effective resistance against aging-associated skin xerosis.

The invention of Gueniche et al. (2006a, b) related to the use of an effective amount of at least one microorganism belonging to the species *L. paracasei* or *casei*, a fraction thereof or a metabolite thereof, in combination with an effective amount of at least one microorganism belonging to the species *Bifidobacterium lactis*, a fraction thereof or a metabolite thereof, for producing a dermatological composition intended for treating and/or preventing reactive, irritable and/or intolerant, acquired dry skin and/or constitutional dry skin.

The cosmetic and/or dermatologic use of hesperidin (a flavonoid) in combination with probiotic microorganism for preventing a reduction in and/or for reinforcing the barrier function of the skin associated with aging and/or photoaging was also proposed in a recent patent of Gueniche and Castiel (2009). The authors also presented an invention related to the cosmetic use of an effective amount of at least one microorganism, especially a probiotic microorganism, or a fraction thereof, as an agent for preventing the appearance and/or for treating the manifestation of sensations of discomfort and/or cutaneous signs associated with a surface skin treatment or an invasive treatment for esthetic purposes (Castiel and Gueniche 2009).

Epidermal keratinocyte differentiation is another essential event that coordinates epidermal turnover and construction of skin barrier function. In this context, Baba et al. (2006) reported that *L. helveticus*-fermented milk was able to promote differentiation of cultured normal human epidermal keratinocytes by enhancing production of the differentiation-related element profilaggrin, a precursor of a natural moisturizing factor that controls normal epidermal hydration and flexibility.

3.3 Hyaluronic Acid

Fermentation technology using lactic acid bacteria was also proposed to produce many other beauty factors, including hyaluronic acid (HA) and collagen, that provide beneficial activities in maintaining skin health and preventing skin aging (Chong et al. 2005; Chiba 2007). In the skin, HA represents a predominant voluminous molecule of extracellular matrix (ECM). It is synthesized by keratinocytes and fibroblasts and performs important biological role in the skin by having specific rheological characteristics and good water-holding properties. HA has several applications in medicine and cosmetics, including skin moisturizers. The ability of *Bifidobacterium*-fermented soy milk extract (BE) to significantly enhance HA synthesis in vitro and in vivo has also been performed (Miyazaki et al. 2003, 2004). The authors established that topical application of BE was able to ameliorate the elasticity and viscoelasticity of mouse skin, to increase HA level and thus preventing the age-dependent loss of cutaneous HA. The topical application of a gel formula containing BE on human skin significantly enhanced skin elasticity suggesting BE as a new cosmetic ingredient to improve skin elasticity through augment of

HA production. Recently, Izawa et al. (2010) described the optimized fermentation conditions in a skimmed milk-based medium which had significantly (about 20-fold) increased HA yield from *S. thermophilus* YIT 2084. Because of the safety of this bacterium, the fermentation technique would have an impact on the application of the bacterial HA.

3.4 Collagen

Collagen is a major constituent of the human skin and accounts for a high proportion of the skin's elasticity and physical properties. It is well documented that exposure to sunlight damages the skin structures. In response to this damage, the skin repairs itself through the rapid production of collagen and other associated dermal components such as polysaccharides. A recent invention of Lieurey and Watkins (2009) reported that the use of a fermented milk product comprising nonhydrolysed and casein-free whey proteins improved skin firmness when topically applied to skin. The milk fermented with classic lactic acid bacteria (*S. thermophilus* and *L. bulgaricus*) could improve the structuring of skin's collagen without promoting collagen synthesis. The inventors suggested the topical use of the fermented milk on damaged skin to improve the collagen natural generation as part of the repair process, thus preventing a skin condition termed elastosis.

4 Probiotics and Skin Inflammatory/Immune System

Skin consists of stratified epithelium with various cell types, including keratinocytes that have been specialized to act as the outpost of the innate defense system and, in a lower proportion, dendritic cells, melanocytes, and Langerhans cells. Each of these cell types contributes to skin protection. Moreover, the underlying dermal compartment harbors leukocytes, mastocytes, and macrophages that are key actors of cell defense. Probiotics actions on the skin can be mediated by modulation of host's immune response including innate as well as adaptive.

4.1 Barrier Function and Skin Reactivity

Some probiotic strains display potent immune-modulatory properties at the skin level. Recently, the ability of *L. paracasei* CNCM-I 2116 (ST11) to modulate reactive skin-associated inflammatory mechanisms has been evaluated (Gueniche et al. 2010a). The authors showed that ST11 was able to abrogate vasodilation, edema, mast cell degranulation, and TNF-alpha release which induced by substance P, compared to control. Moreover, using ex vivo skin organ culture, the authors showed that ST11-conditioned medium induced a significantly faster barrier

function recovery after sodium lauryl sulphate disruption, compared to control. These results support a beneficial role of ST11 on key biological processes associated with barrier function and skin reactivity. Gueniche et al. (2010b) performed an in vitro and a clinical trial using *B. longum* sp. extract proved that these nonreplicating bacteria forms applied to the skin were able to improve sensitive skin in various parameters associated with inflammation such as decrease in vasodilation, edema, mast cell degranulation, and TNF-alpha release. These findings suggest that new approaches, based on a bacteria lysate, could be developed for the treatment and/or prevention of symptoms related to reactive skin. A recent invention of Gueniche (2010) disclosed methods directed to the cosmetic use of an effective amount of at least one probiotic microorganism especially from the genus *Lactobacillus* and/or *Bifidobacterium*, or a fraction thereof and/or a metabolite thereof, as an active agent for limiting, preventing or treating skin irritation, and/or irritative skin disorders.

The innate immune system uses pattern recognition receptors such as Toll-like receptors (TLRs) to recognize microorganisms or their products on the cell membranes (Guan and Mariuzza 2007). Keratinocytes were found to express TLR2, which is specifically involved in the recognition of peptidoglycan (PGN), a mesh-like layer outside the plasma membrane of bacteria forming the cell wall that protects bacteria from environmental stress. It has been recently demonstrated that TLR2 can differentially recognize PGN from Gram-positive and Gram-negative bacteria (Asong et al. 2009). DAP-containing muropeptides were bound with high affinity to TLR2, whereas only a restricted number of Gram-positive lysine-containing muropeptides, derived from PGN remodeled by bacterial autolysins, were recognized. The difference in recognition of the two classes of muropeptides is proposed to be a strategy by the host to differentially respond to Gram-negative and Gram-positive bacteria, which produce vastly different quantities of PGN. Lactobacilli were demonstrated to stimulate innate immune response via TLR2 and nucleotide-binding oligomerization domain-2 thus modulating dendritic cell function (Zeuthen et al. 2008). They were also demonstrated to induce the production of interleukin-12 and other regulatory factors by macrophages (Sun et al. 2005; Shida et al. 2006; Bleau et al. 2007). Most notably, *L. casei* strain Shirota, both as intact cells or as cell wall-derived polysaccharide–peptidoglycan complex (PSPG), inhibited IL-6 production in lipopolysaccharide (LPS)-stimulated lamina propria mononuclear cells isolated from murine models of inflammatory bowel disease and induced an improvement of disease conditions in mice (Matsumoto et al. 2009). In the light of these findings, it is reasonable to hypothesize a possible role of *Lactobacillus* surface molecules in modulating inflammatory response in skin.

4.2 Environmental Stress

Skin is the largest human organ and is directly exposed to environmental stress which may cause oxidative stress, especially UV irradiation. Indeed, UVB (290–320 nm) and UVA (320–400 nm) light can both induce the generation of reactive oxygen

and nitrogen species (ROS/RNS) in human skin (Xu and Fisher 2005). When the generation of ROS/RNS exceeds the skin antioxidant defenses, the consequence is epidermal oxidative stress. Excess production of ROS/RNS may affect the cell function leading to apoptotic or necrotic cell death. Oxidative stress is thought to play a central role in initiating and driving the signaling events that lead to cellular response following skin UV irradiation. Increased ROS/RNS production induced by UV light alters gene and protein structure and function, leading to skin damage through the activation of multiple cytokine and growth factor cell surface receptors (Rittié and Fisher 2002). Many of the molecular alterations observed following UV irradiation of skin also occur during aging, a condition which is known to be associated with oxidative stress. Indeed, it is well known that endogenous antioxidants are decreased in skin and blood during UV exposure and in senescence (Rittié and Fisher 2002). Hence, a treatment aimed at counteracting oxidative stress may be helpful in the prevention of damages caused by UVB and UVA light or skin aging. Furthermore, epidemiological studies clearly indicate UV light exposure as the major cause of skin cancer (Armstrong and Kricker 2001); hence it is recommended to adopt preventive measures by sunscreens via the topical route, in addition to antioxidants via the systemic route. A number of reports indicate that food supplementation with antioxidant molecules (such as vitamins C and E, carotenoids, flavonoid, polyphenols, thiol compounds, and selenium) is able to counteract skin damage by UVA and UVB (Greul et al. 2002).

Many evidences indicate that probiotics may be helpful as antioxidant agents, both in vitro and in vivo. A number of probiotic strains were demonstrated to possess antioxidative action in vitro. Lin and Yen (1999) and Lin and Chang (2000) demonstrated that various *Lactobacillus* and *Bifidobacterium* strains were able to exert antioxidant action in vitro. Both intact cells and cell-free extracts were able to inhibit ascorbate autoxidation, to exert metal-chelating ability, to scavenge superoxide anion and other ROS, and to inhibit lipid peroxidation. Probiotics' ability to act as antioxidant can be attributed to the presence of antioxidant enzymes such as superoxide dismutase (Shen et al. 2010) to the release of antioxidant compounds such as glutathione (Peran et al. 2006) and to the production of extracellular polysaccharide (EPS) biomolecules that probiotic bacteria release into the surroundings to protect themselves under starvation conditions and also from extreme pH and temperature conditions (Kodali and Sen 2008). Ingestion of probiotics may also exert systemic protection from oxidative stress. Lactic acid bacteria extracts or fermented milks were found to decrease human low-density lipoprotein oxidation and to prolong the resistance of the lipoprotein fraction to oxidation (Terahara et al. 2001; Kullisaar et al. 2003; Gueniche et al. 2006b, 2008; Peguet-Navarro et al. 2008; Bouilly-Gauthier et al. 2010). Thus, probiotics may represent a useful therapeutic tool for the prevention of epidermal oxidative stress either via the topical route or via ingestion. On the basis of these premises, our group has recently performed a number of experiments in order to study the antioxidant activity of a specific strain of lactic acid bacteria, the *S. thermophilus* S244 (provided by VSL Pharmaceuticals, Inc., Gaithersburg, MD, USA) (manuscript in preparation). Extracts of this bacterium were found to be good free radical scavengers, as

compared to Trolox used as reference antioxidant compound. The oxygen radical absorbance capacity of the bacterial lysate, measured as the area under the curve, revealed that the bacterial extract efficiently inhibited the free radical-dependent loss of phycoerythrin-E fluorescence in a dose-dependent fashion (not shown). Encouraged by these results we assessed the photoprotective activity of the bacterial lysate. When human HaCaT keratinocytes were irradiated with UVB light, a 50% reduction in cells viability was observed. However, when the cells were treated with UV in the presence of the bacterial extract, a dose-dependent protection of cell viability was observed (not shown). In the light of our data on the antioxidant activity of bacterial extracts, we propose that the photoprotective effect may be at least in part due to the good free radical-scavenging properties of the extract.

4.3 Protection Against NO

The NO pathway has been shown in several cell types that reside in the skin, including keratinocytes, melanocytes, Langerhans cells, fibroblasts, and endothelial cells (Bruch-Gerharz et al. 1998). Convincing evidence suggests that NO synthesis in these cells can be modulated by calcium-mobilizing agonists as well as diverse inflammatory and immune stimuli, and thereby contributes to the pathogenesis of several human skin diseases. Characterization of these intrinsic and extrinsic regulatory stimuli of NO synthesis has afforded substantial insights into the role of NO in inflammatory, hyperproliferative, and autoimmune skin diseases, as well as skin cancer, and may ultimately form the basis for future therapeutic intervention. NO is synthesized from arginine and oxygen by various nitric oxide synthase (NOS) enzymes. NOS is a group of enzymes responsible for the synthesis of NO from the terminal nitrogen atom of L-arginine in the presence of oxygen and some cofactors. The presence of arginine deiminase in *L. brevis*, previously reported by our group (Di Marzio et al. 2001), was further characterized through activity and expression studies. *L. brevis* arginine deiminase, being able to metabolize arginine to citrulline and ammonia, allows to inhibit NO generation by competing with NOS for the same substrate, arginine. Considering the role of NO in inflammatory conditions our group had also analyzed the ability of *L. brevis* to inhibit NOS activity as well other inflammatory parameters including IFN-γ and PGE_2 release and MMP expression in murine macrophages activated by LPS (Della Riccia et al. 2007). The results suggested that the presence of *L. brevis* extracts in cell culture strongly inhibited inducible NOS activity, IFN-γ/PGE_2 production, and MMP activity in LPS-activated macrophages. These effects could be attributed to *L. brevis*' ability to prevent inducible NOS activity responsible for NO, a key inflammatory molecule. The invention of De Simone (2003) disclosed the use of bacteria endowed with arginine deiminase to induce apoptosis and/or reduce an inflammatory reaction, and pharmaceutical compositions containing such bacteria, including creams and ointments. The inventor also included a strain of *L. brevis* referred to as CD2 highly endowed with arginine deiminase.

4.4 Anti-inflammatory Potential

In the context of the anti-inflammatory potential of probiotics, the in vitro and in vivo effects of supernatants from *L. acidophilus* (ATCC strains 4356 and 43121) on tissue repair and angiogenesis were investigated by Halper et al. (2003). The authors suggested that *Lactobacillus* supernatant promoted proinflammatory processes including chemoattraction of polymorphonuclear cells, macrophages, and angiogenesis in addition to previously described stimulation of production of TNFα and other cytokines including interleukins and interferons.

Trautmann et al. (2001) demonstrated that in atopic dermatitis and allergic contact dermatitis, skin-activated T cells stimulated Fas-induced keratinocyte apoptosis. In particular, diseased skin-infiltrating T cells produce IFN-γ that increasing Fas receptor number on keratinocyte membrane renders them susceptible to apoptosis by Fas ligand expressed on or released by T cell surface. The knowledge of these mechanisms provided a useful molecular model to focus on innovative therapeutic applications. In order to examine the role of probiotics on inflammatory skin disease, our group investigated the effect of a selected extract from *B. infantis* on human keratinocyte cell line (HaCaT) abnormal apoptosis induced by activated T-lymphocyte (Cinque et al. 2006). In particular, the probiotic effect on inflammatory skin disease was investigated in the experimental model of AD as proposed by Trautmann (2001). In this in vitro model of atopic AD, the ability of the probiotic extract to protect HaCaT from apoptosis induced by soluble factors (IFN-γ and CD95 ligand) released by human T-lymphocytes in vitro, activated with anti-CD3/CD28 mAbs or phytohemoagglutinin, has been evaluated. The obtained results highlighted the bacterial extracts' ability to totally prevent T-lymphocyte-induced HaCaT cell apoptosis in vitro. The mechanism underlying this inhibitory effect has been suggested to depend on the ability of the bacterial extracts to significantly reduce anti-CD3/CD28 mAbs and mitogen-induced T-cell proliferation, IFN-γ generation, and CD95 ligand release. These results may represent an experimental basis for a potential therapeutic approach mainly targeting the skin disorders-associated immune abnormalities.

Cutaneous immune responses must be tightly controlled to prevent unnecessary inflammation in response to innocuous antigens, while maintaining the ability to combat skin-tropic pathogens. Regulatory T cells (Treg) play an important protective role against autoimmune response to self-antigens to maintain self-tolerance, functioning by suppressing the activation, cytokine production, and proliferation of other T cells. Tregs are CD4- and CD25-positive cells but the most specific marker for these cells is FoxP3 (forkhead box P3), which is localized intracellularly. Dysregulation in Treg cell frequency or functions may lead to the development of autoimmune disease. Treg modulation is considered to be a promising therapeutical approach to treat some selected disorders, such as allergies, and to prevent allograft rejection (Clark 2010). In normal human skin these cells represent between 5 and 10% of the T cells resident and proliferating in inflamed skin serving as a brake for cutaneous inflammation. Indeed their number increases in the skin lesions of

contact dermatitis and in DTH reactions (Teraki and Shiohara 2003; Vukmanovic-Stejic et al. 2008). Of note, in a recent study, de Roock et al. (2010) examined the ability of specific probiotic strains to induce Foxp3-positive Tregs from human peripheral blood mononuclear cells (PBMC) in vitro. The authors highlighted a different capacity of tested probiotic strains to stimulate Treg population demonstrating that *L. acidophilus* proved to be the most potent Treg inducing bacterium. Moreover *L. acidophilus*-induced Foxp3 cells were also able to diminish effector T cell proliferation. All examined probiotic strains were tested for the ability to induce cytokine secretion, and in particular the results showed that all bacteria induced a comparable IFN-γ release while IL-17 and IL-13 were produced in low levels, and IL-4 did not detect. Induction of regulatory T cells is an attractive target in the therapy of disorders where an abnormal immune response is present including autoimmune diseases, asthma, and allergy. For this purpose there is a need to increase understanding about specific role of each single bacterium in modulating the Treg function in distinct compartments, both in the intestine and locally in inflamed tissue. Volz and Biedermann (2009) sustained the topical application of probiotics for prophylaxis and therapy of overwhelming cutaneous

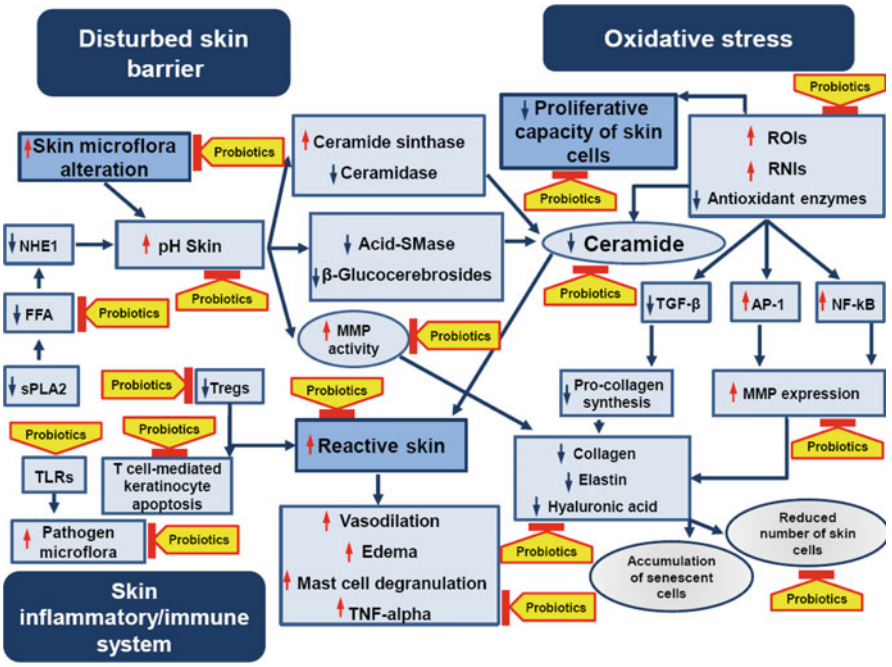

Fig. 1 Comprehensive model that summarizes the main actions carried out by probiotics in different skin conditions associated with altered microflora, abnormal oxidative stress, disturbed skin barrier, and/or inflammatory/immune skin reactions. It is important to note that, for simplicity, the generic term "probiotics" is indicated but it must be implied that the claimed effect should be attributed to specific species or specific strains of probiotics as indicated in the text

proinflammatory immune reactions, considering it very promising also on the basis of the ability of specific probiotic strains to trigger the production of tolerogenic IL-10 by activating anti-inflammatory Treg.

5 Conclusions

Topical probiotic formulations are becoming increasingly available for healthy skin care, prevention and treatment of skin diseases, and antiaging benefits, thus representing an emerging area for skin health. The potential benefits of skin probiotics could strongly depend on how each species or strain is selected as the specific mechanisms underlying a specific effect on the healthy or disturbed skin. It appears, therefore, particularly important to stress that it is not possible to generalize the effects that each of them, any association or combination thereof or extracts thereof, has on the skin. A comprehensive model that summarizes the main actions carried out by probiotics in different skin conditions associated with altered microflora, abnormal oxidative stress, disturbed skin barrier, and/or inflammatory/immune skin reactions is shown in Fig. 1.

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